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**Shimizu et al.**

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(54) **CALCIUM-INDEPENDENT  
PHOSPHOLIPASES A<sub>2</sub>, GENES THEREOF  
AND PROMOTER OF THE SAME**

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(52) **U.S. Cl.** ..... **435/198; 435/18; 530/350**

(58) **Field of Classification Search** ..... **435/198,  
435/18; 530/350**

See application file for complete search history.

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PLLC

(57) **ABSTRACT**

Novel calcium-independent phospholipases A<sub>2</sub>; genes  
encoding the same; an antibody against them; an inherent  
promoter or a regulator gene which comprises a base  
sequence occurring in intron and inducing site-specific  
expression in response to an external stimulus; a method of  
expressing a target protein in response to an external stimu-  
lus; and an organism having this gene transferred thereinto.  
Novel calcium-independent phospholipases A<sub>2</sub> having an  
amino acid sequence represented by SEQ ID NO: 1, 3 or 5  
or an amino acid sequence derived from such an amino acid  
sequence by the substitution, deletion or addition of one or  
more amino acids; a gene having a base sequence occurring  
in an intron and being capable of initiating RNA transcrip-  
tion due to an external stimulus such as a stimulus with  
kainic acid or an electrical stimulus; a method of regulating  
expression by using the gene; and an organism having the  
gene transferred thereinto.

**1 Claim, 15 Drawing Sheets**

FIG. 1

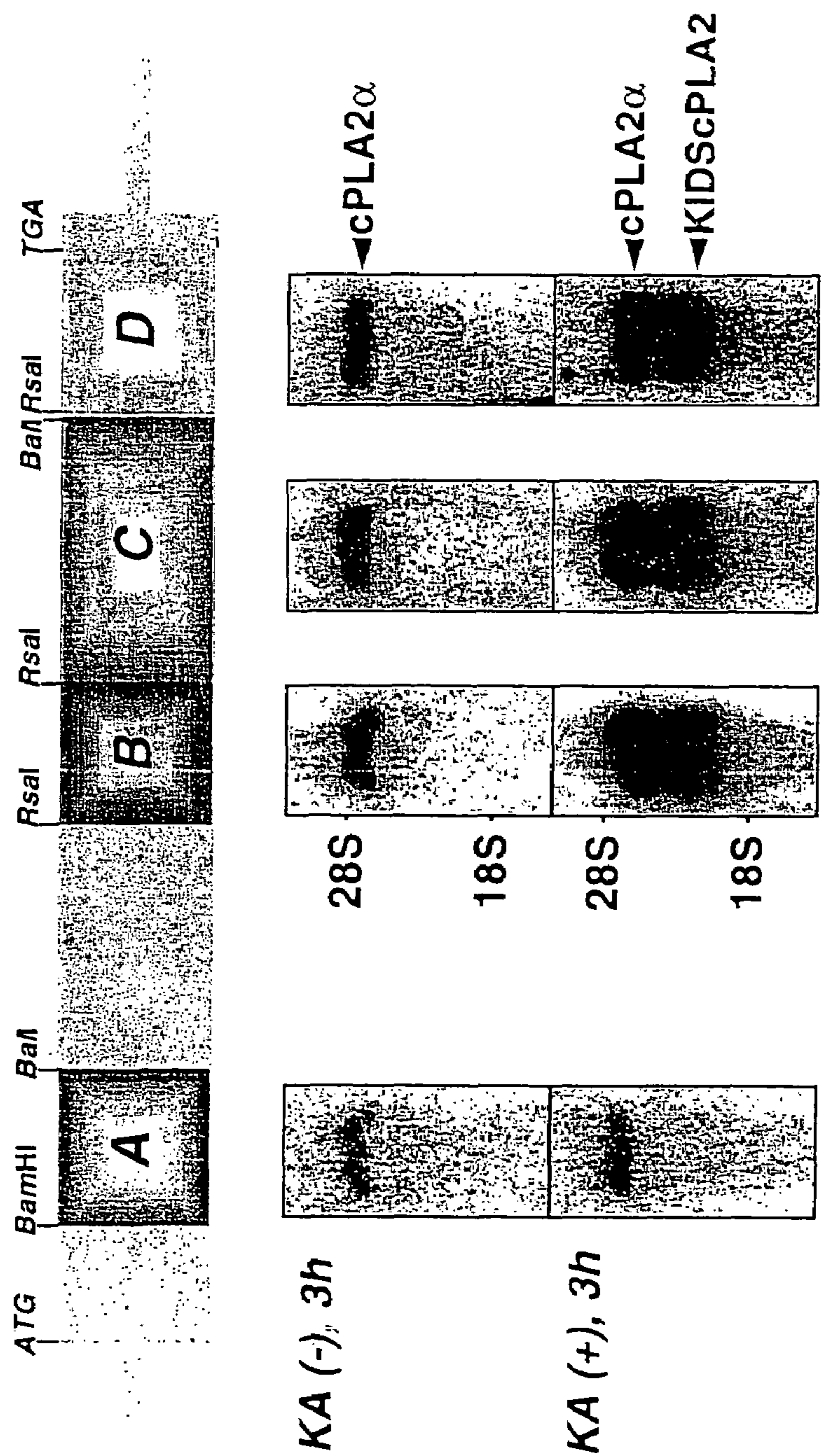




FIG.2

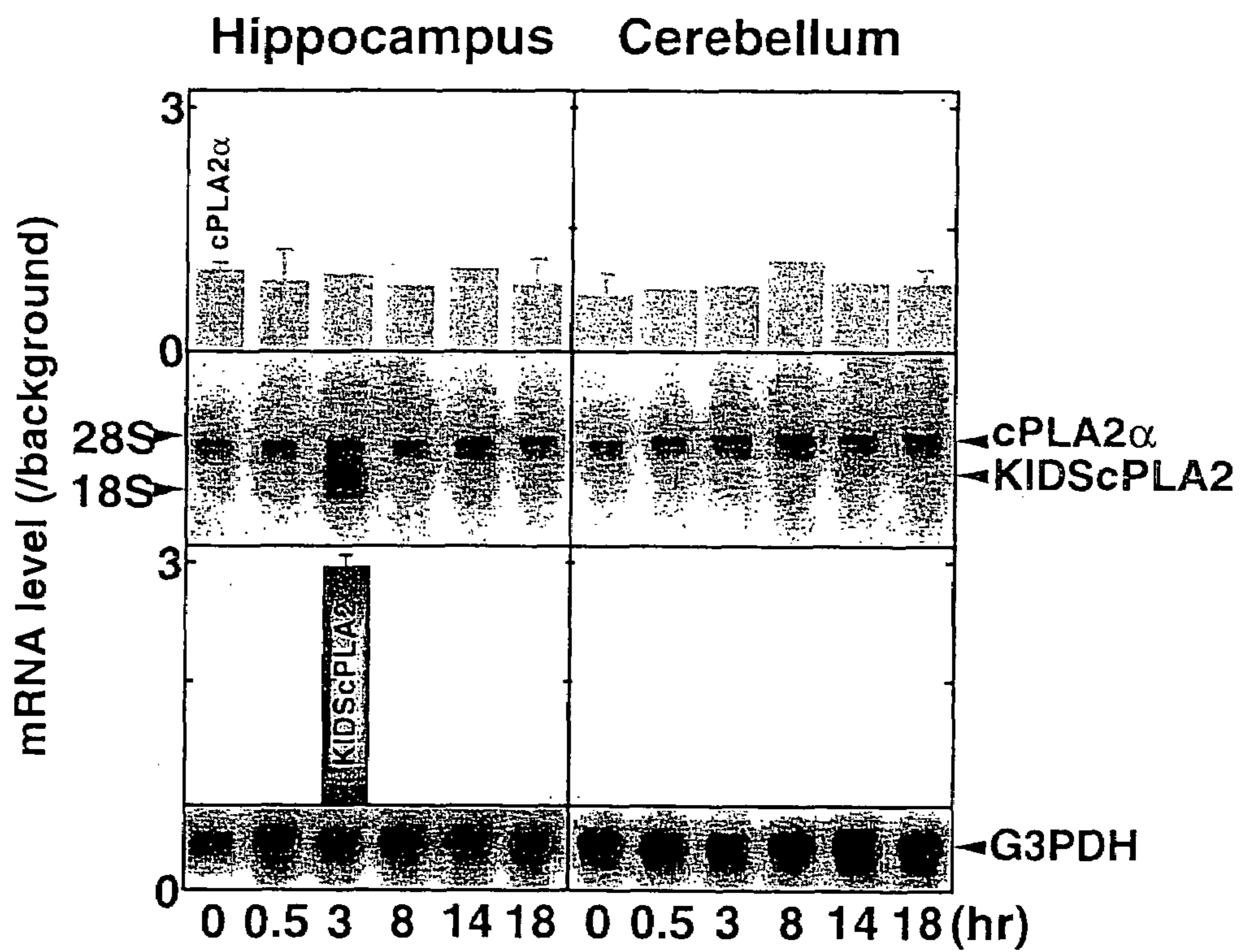
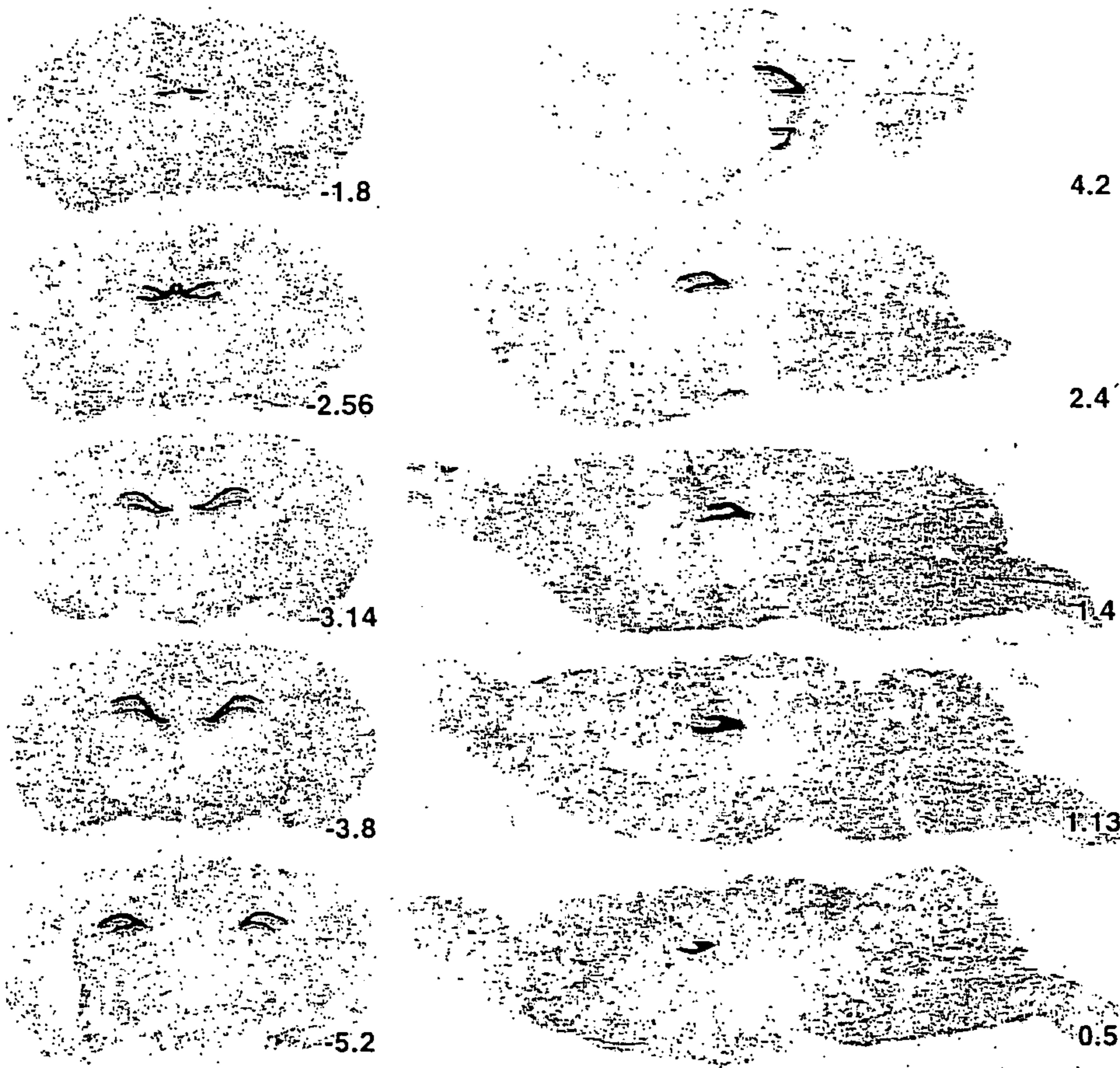


FIG.3

*KA(+), 3h*



*KA (-), 3h*

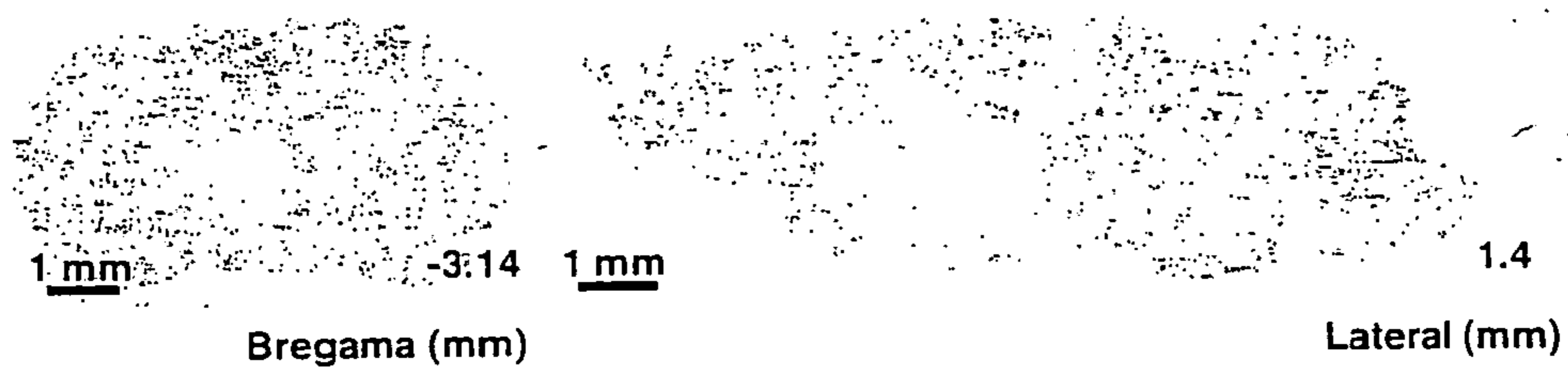




FIG.4

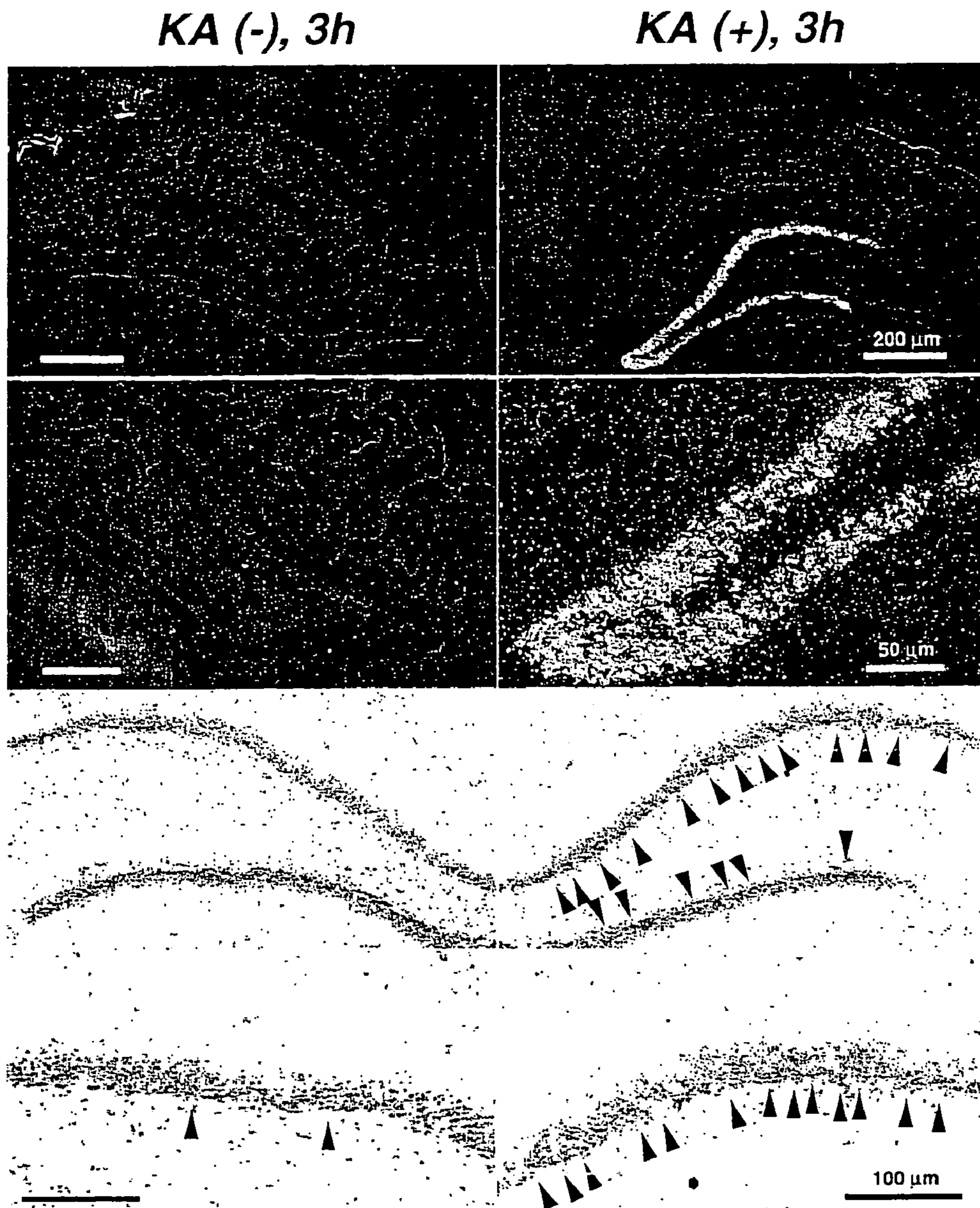


FIG.5

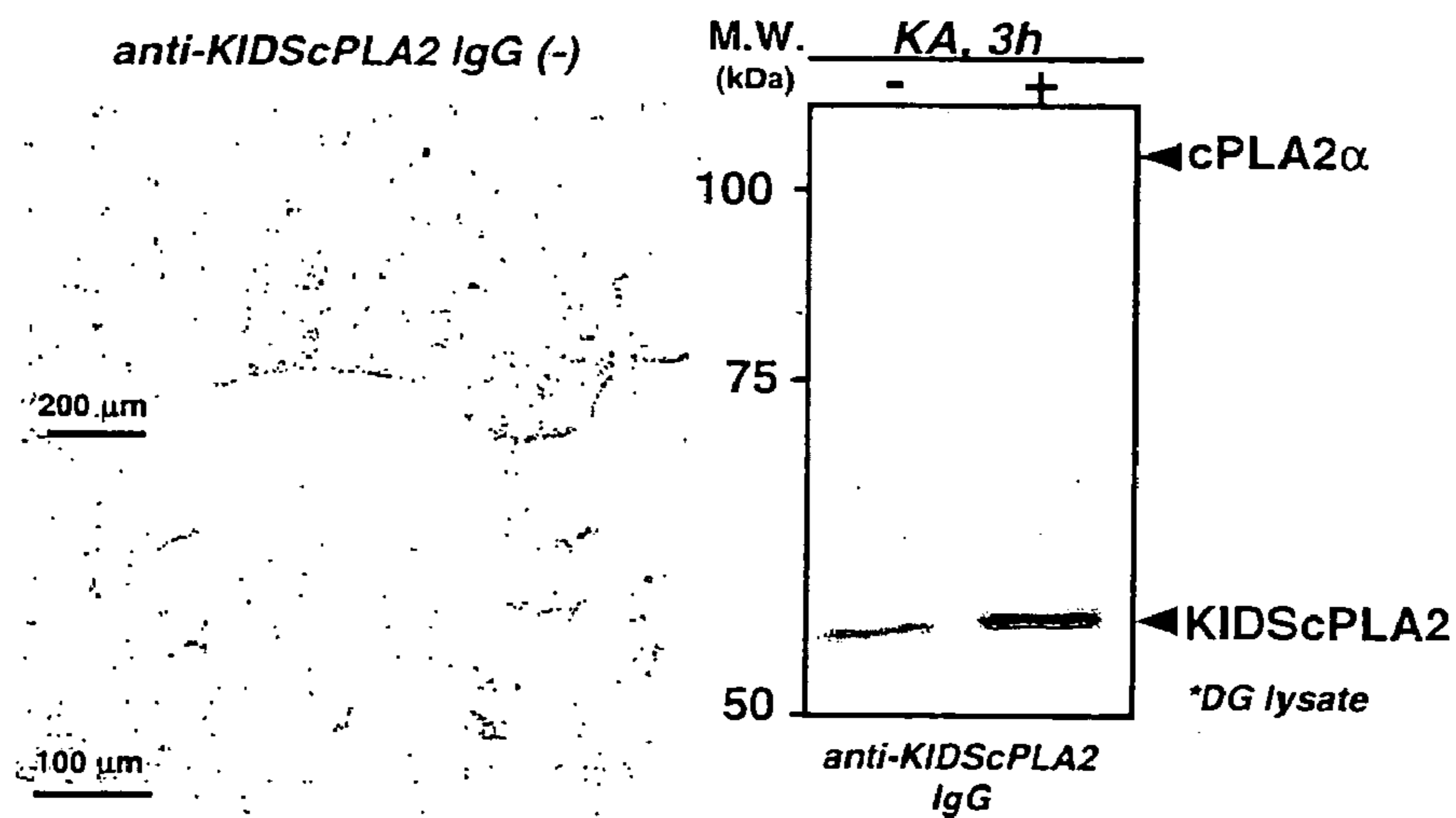
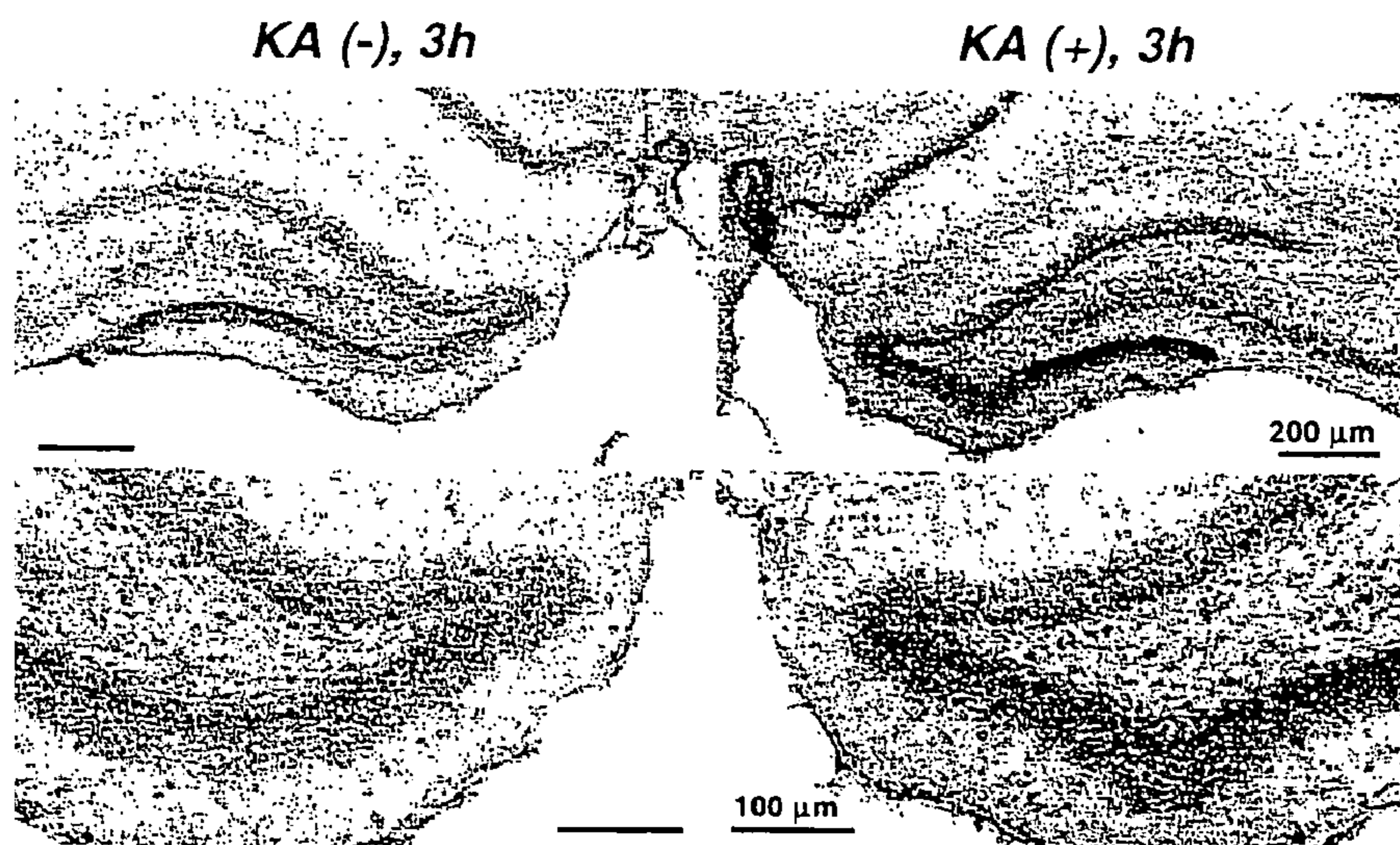


FIG.6

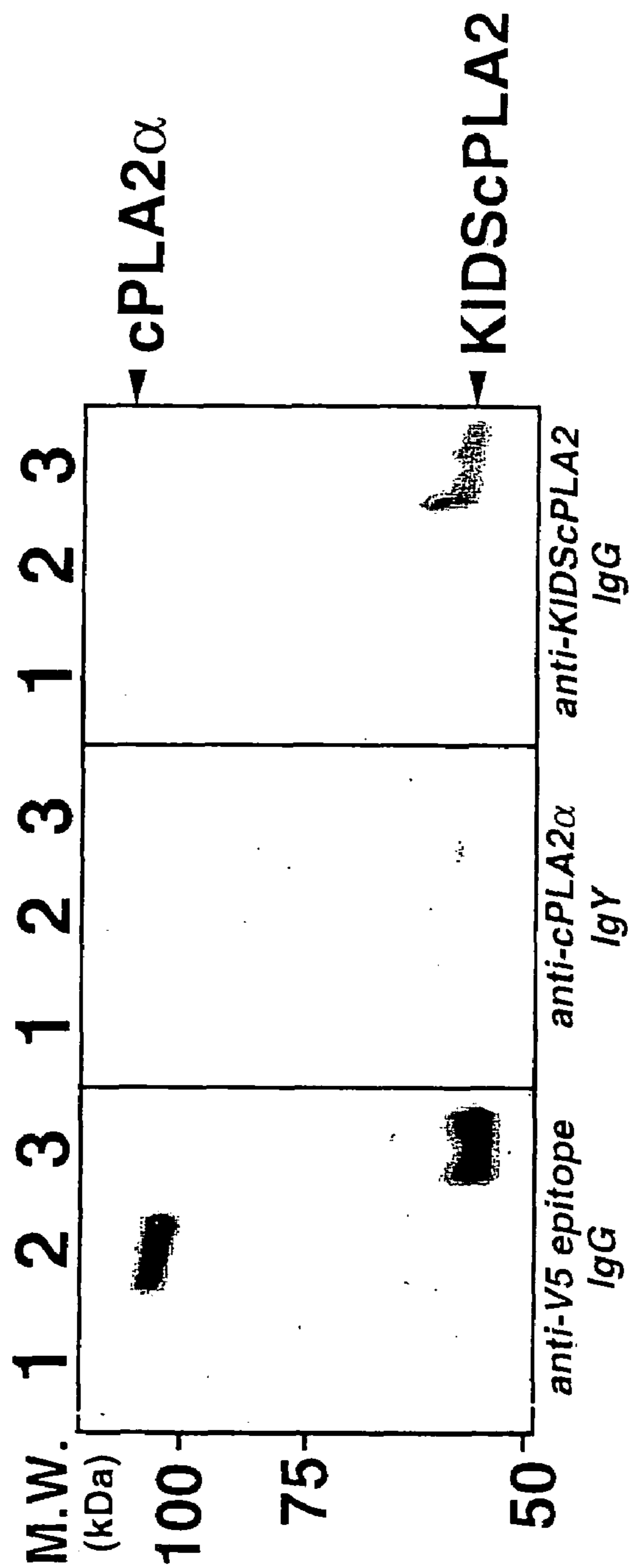




FIG. 7

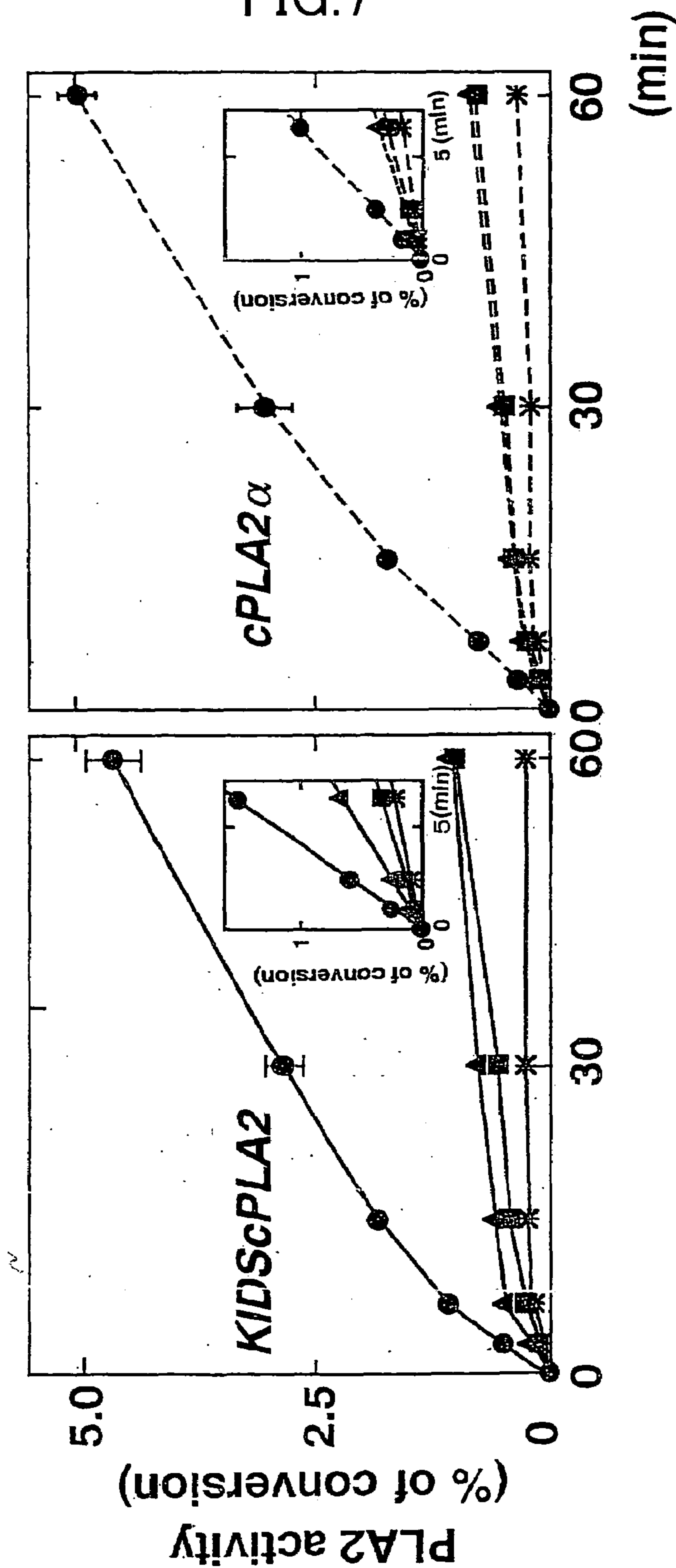




FIG.8

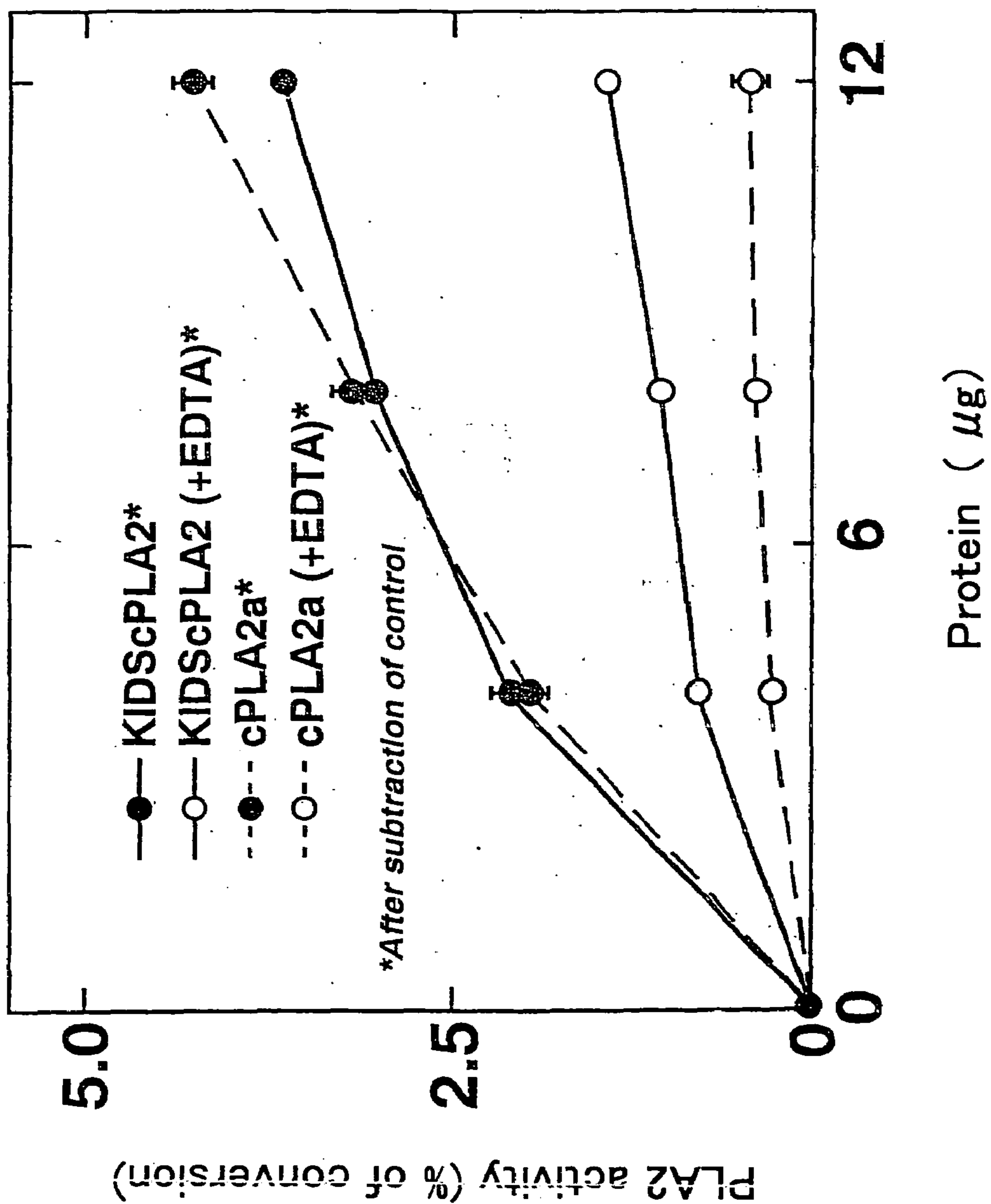


FIG.9

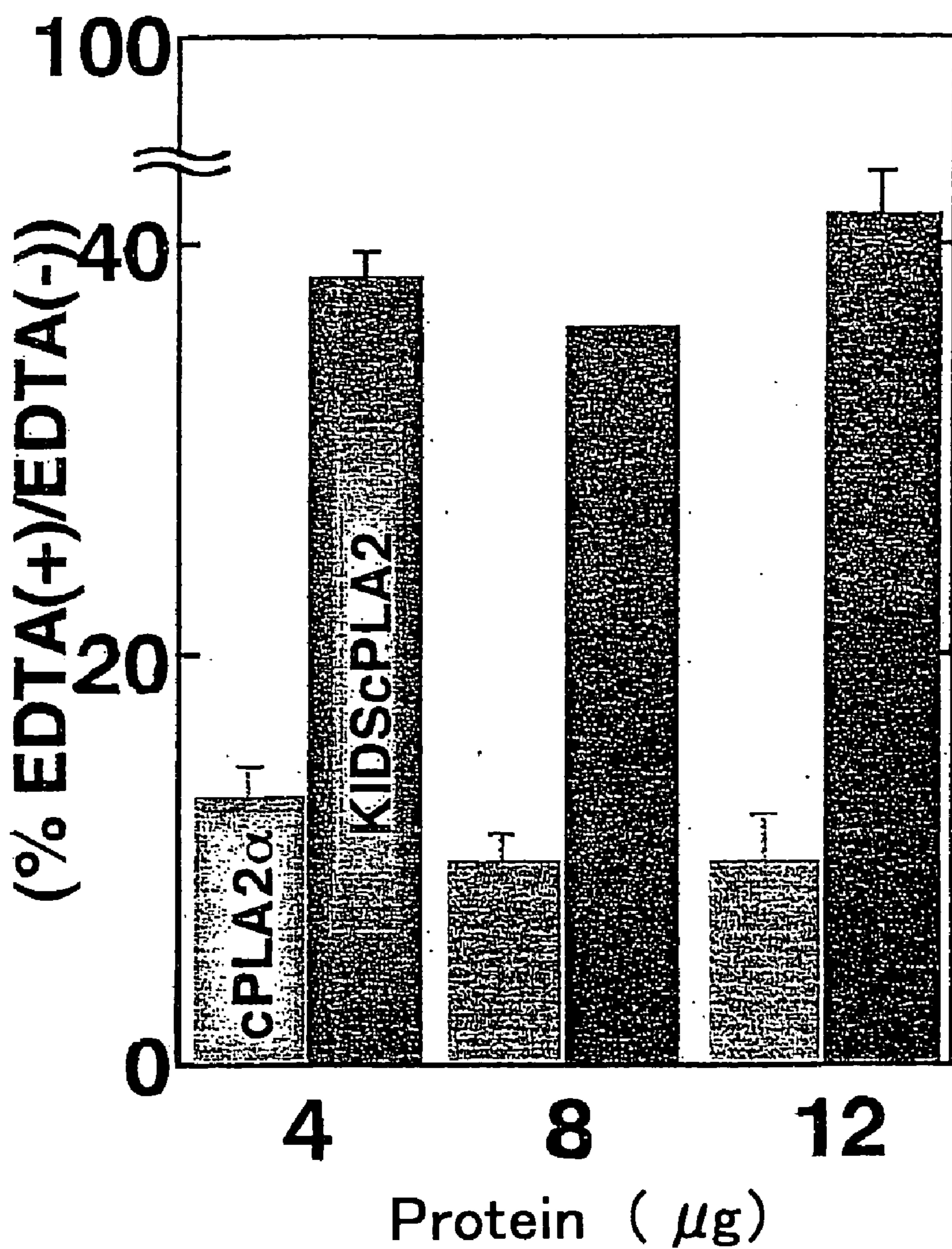




FIG. 10

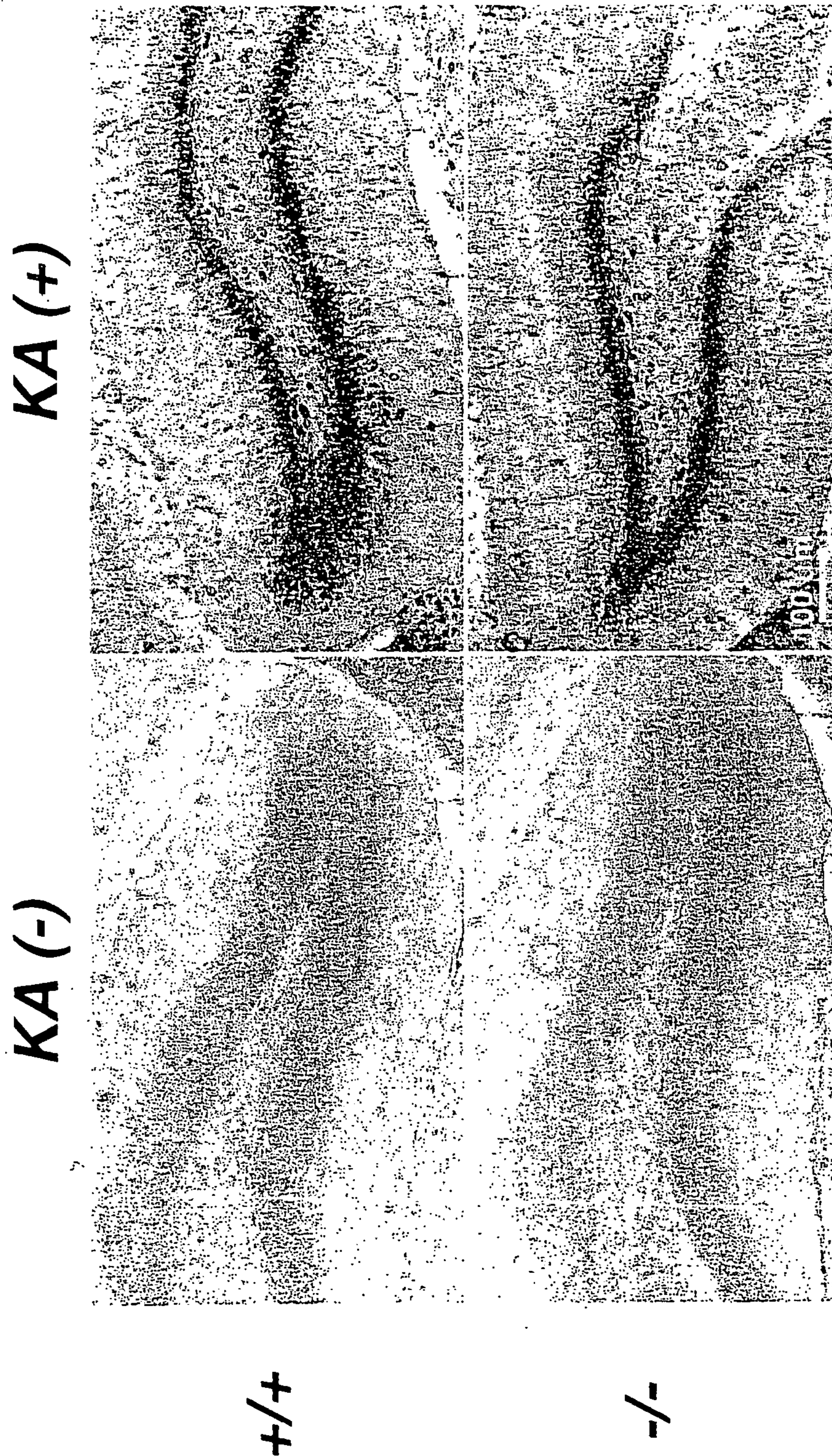
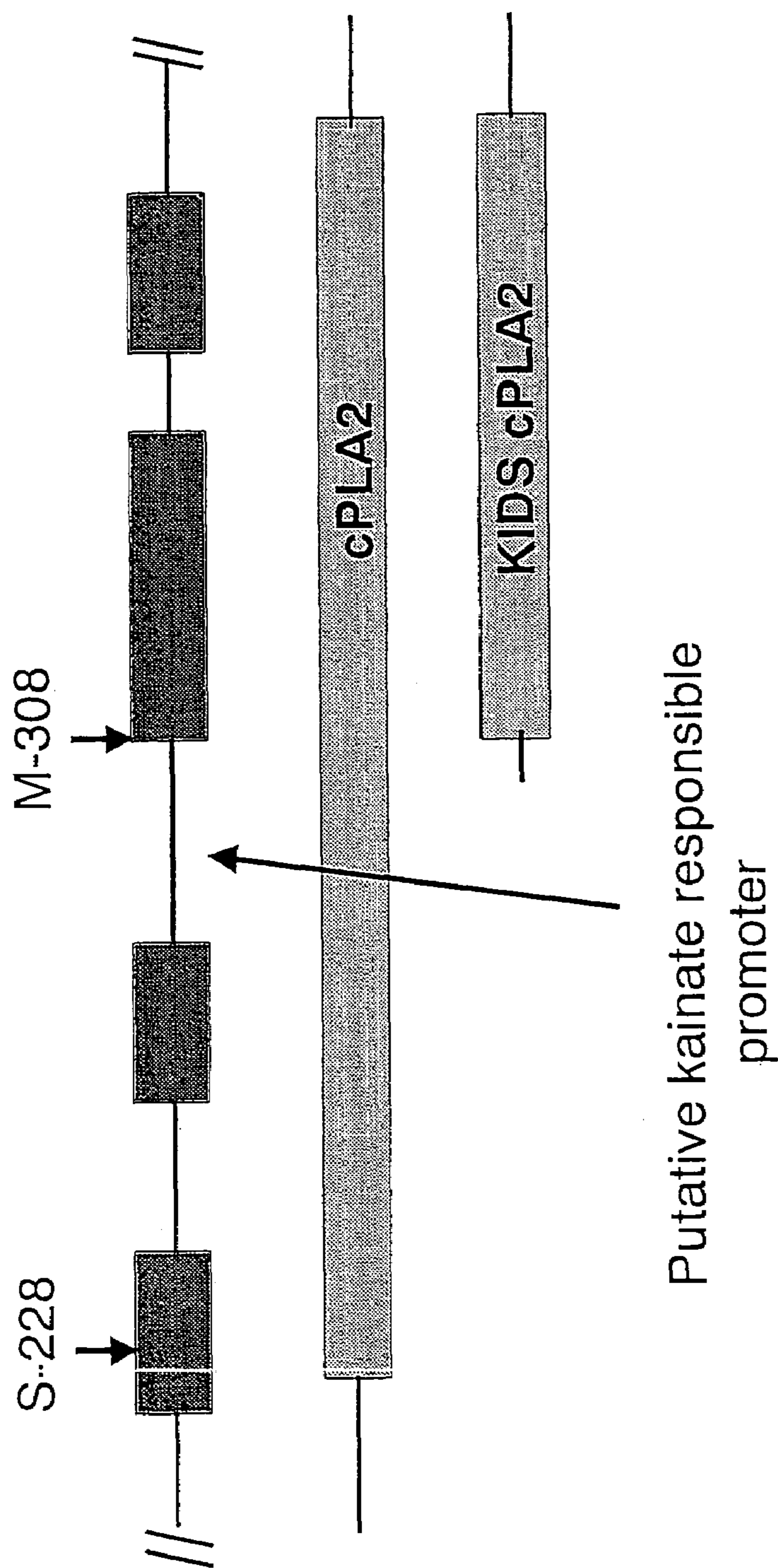




FIG. 11





Rat  
Mouse  
Human

FIG. 12

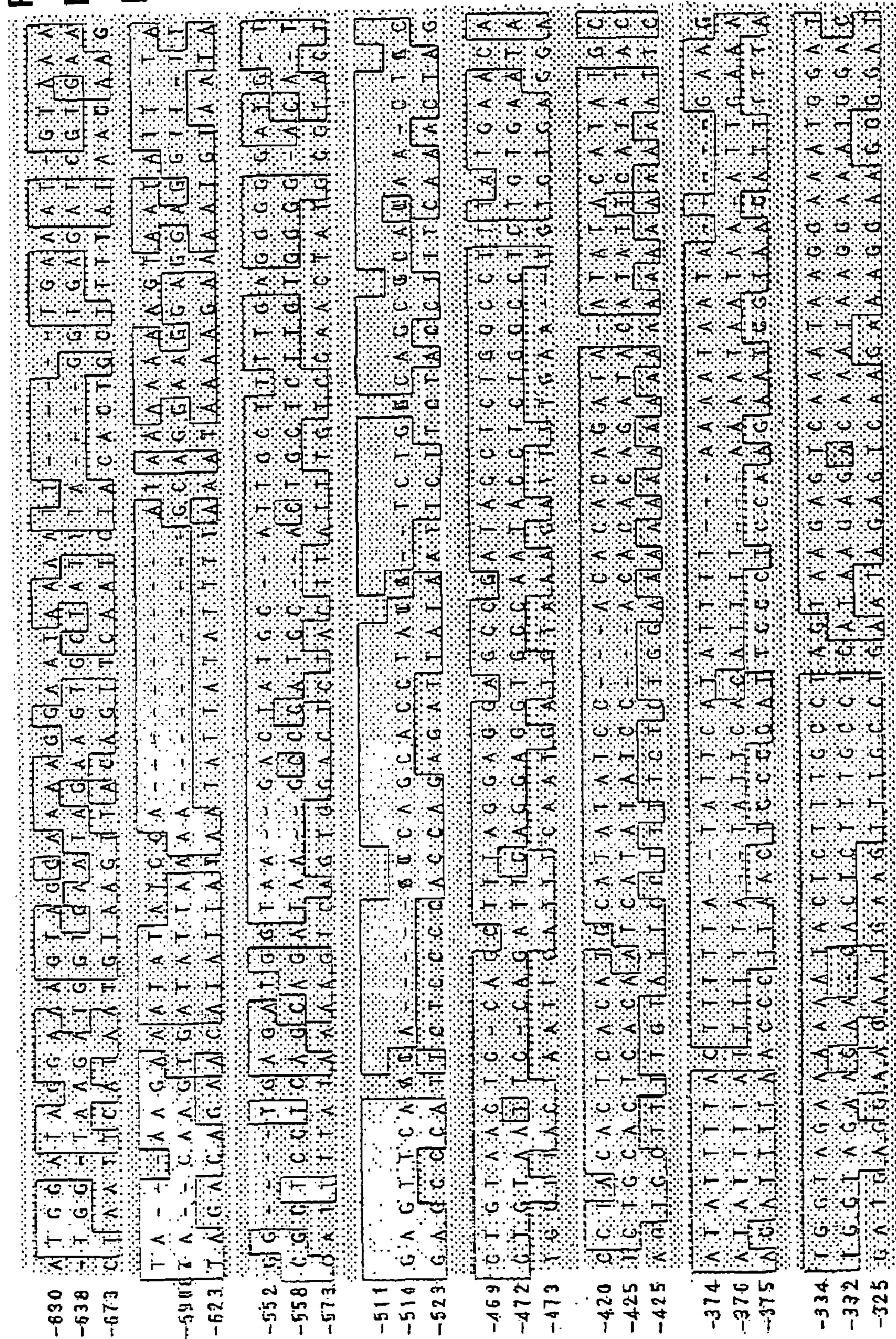




FIG.12 (CONTINUATION)

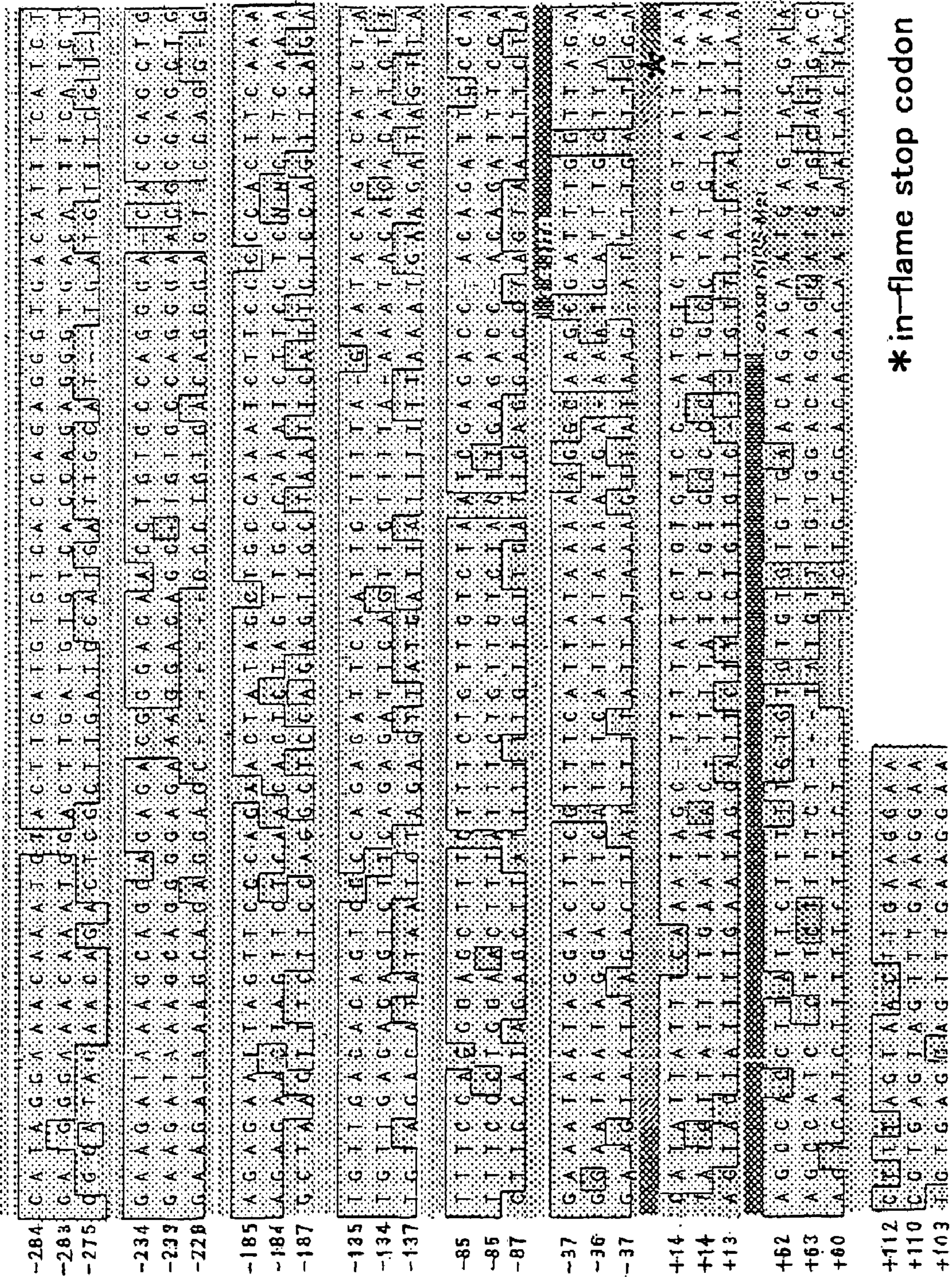
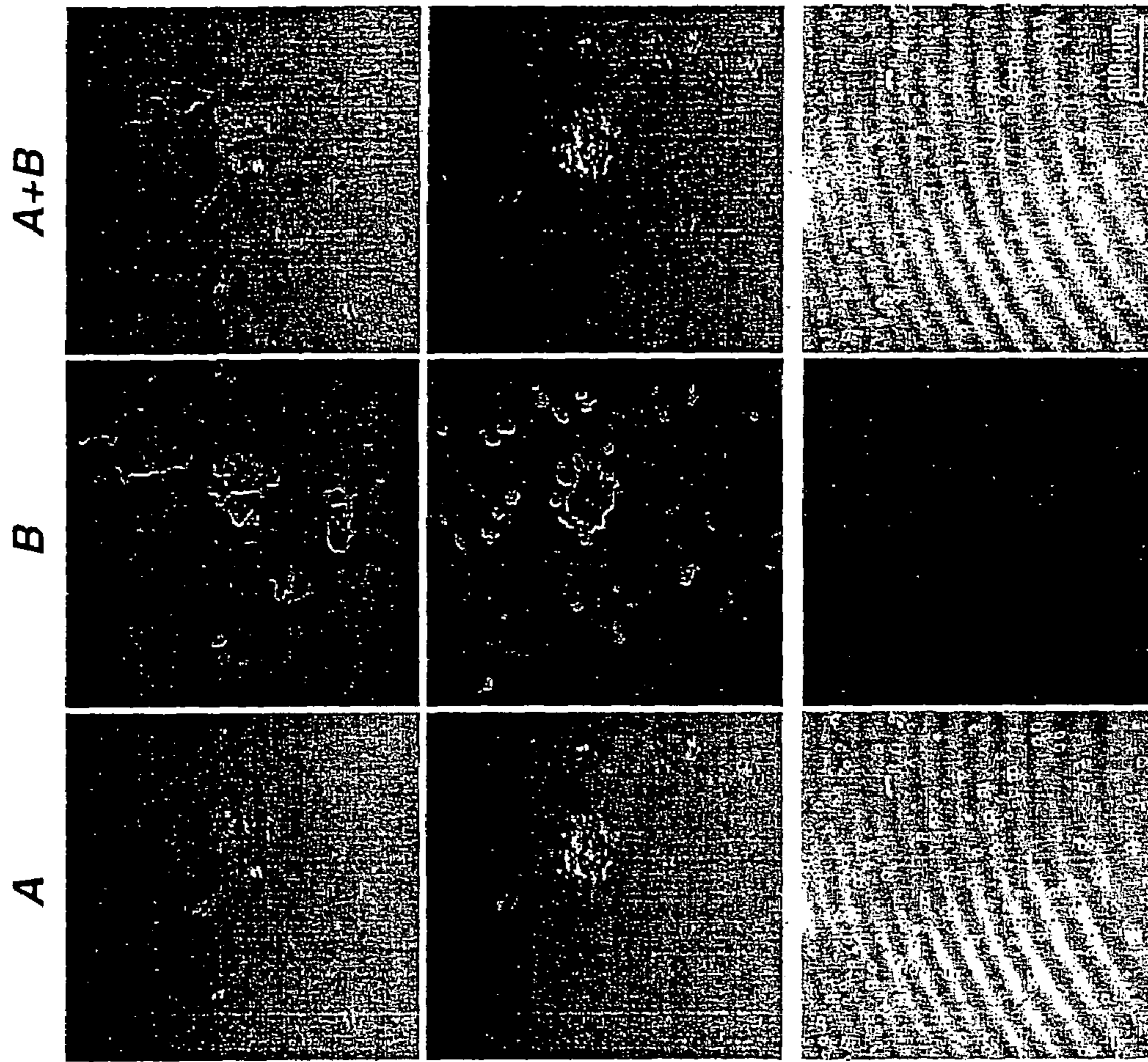




FIG. 13

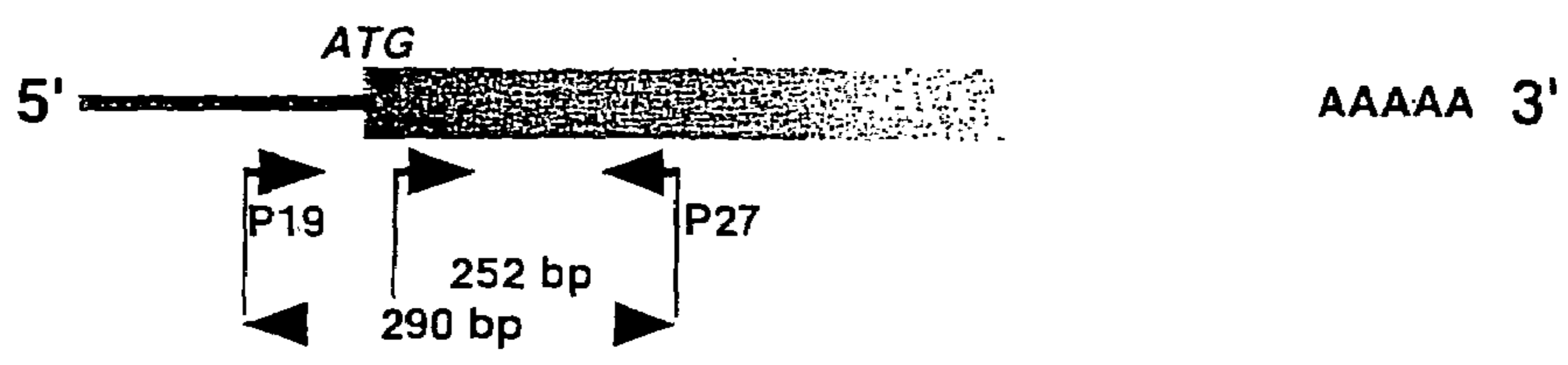
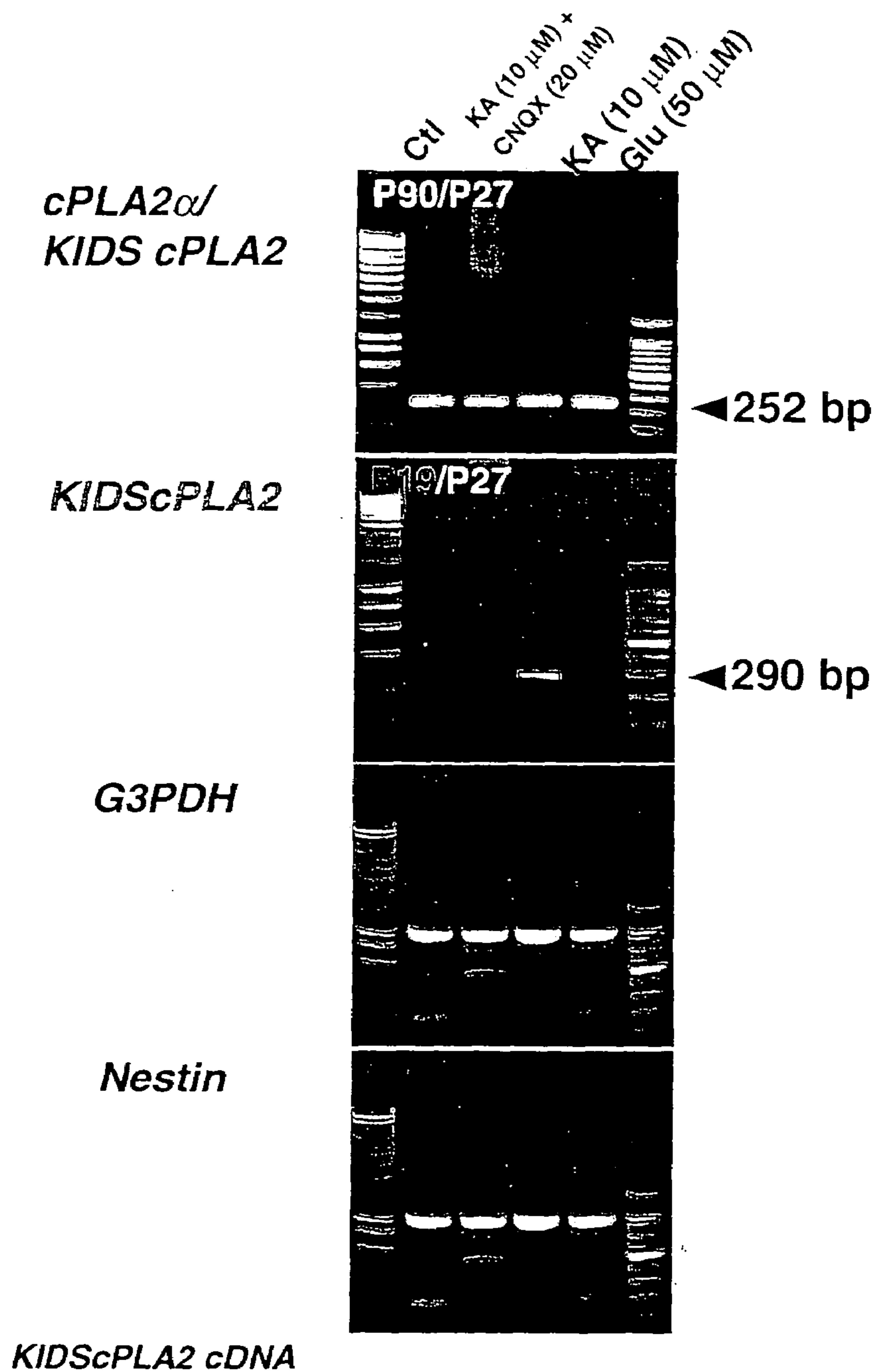


*anti-Nestin (+)*

*progenitor cells*

*mature cells*

FIG.14





1

**CALCIUM-INDEPENDENT  
PHOSPHOLIPASES A<sub>2</sub>, GENES THEREOF  
AND PROMOTER OF THE SAME**

This application is a 35 U.S.C. 371 National Stage entry of PCT Application Ser. No. PCT/JP01/06071, filed Jul. 13, 2001, that claims the benefit of foreign priority under 35 U.S.C. 119(a)-(d) from Japanese Application No. 2001-045938, filed Feb. 22, 2001, the entireties of which are incorporated herein by reference.

TECHNICAL FIELD

The present invention relates to a calcium-independent novel phospholipase A<sub>2</sub> (having a phospholipase A<sub>1</sub> activity as well) and, more particularly, it relates to a novel phospholipase A<sub>2</sub> which is a calcium-independent phospholipase A<sub>2</sub>, is a phospholipase A<sub>2</sub> expressed specifically in hippocampus by an external stimulation such as stimulation by kainic acid or electric stimulation and has an amino acid sequence described in SEQ ID NO: 1, NO: 5 or NO: 8 of the Sequence Listing or an amino acid sequence where one or more amino acid(s) in the amino acid sequence is/are substituted with other amino acid(s) or deficient or one or more amino acid(s) is/are added thereto.

The present further relates to gene having a base sequence existing in intron where the base sequence is able to make the initiation of transcription of RNA specifically to hippocampal dentate gyrus by external stimulation such as kainic acid stimulation or electric stimulation possible, to a method for regulating the expression using the same and to a living thing into which the same is introduced.

BACKGROUND OF THE INVENTION

In the gene of eukaryotes, there are many cases where genetic information stipulating the amino acid sequence of protein is interrupted. A moiety having the genetic information of amino acid sequence of protein is called exon while a moiety having no genetic information of amino acid sequence is called intron. After an mRNA precursor is formed by a transcription of genetic DNA, it is subjected to a splicing so that an intron moiety is cut off whereby mature mRNA is resulted.

It has not been clarified yet why such an intron moiety is present in eukaryotes. However, it has been presumed that, in many cases, one exon is coded as a specific domain (functioning region) of protein and, when new protein having the same function is needed during the process of development, necessary protein is able to be produced by a combination of different exons.

With regard to a splicing of the mRNA precursor before being subjected to the splicing, there has been also known the case where not only intron is cut off but also exon moiety is cut off to give mRNA coding for different protein having the function of the same type.

For example, calcitonin gene has six exons—A, B, C, D, calcitonin CCP and CGRP (calcitonin gene related peptide). Exon A and exon B are non-translated region while translated region is other four exons. When a transcription is carried out in nucleus of cell, all exons are included but a process of the splicing varies depending upon organs. For example, in thyroid C cells, exon of the sixth CGRP is also spliced and, as a result, protein of the translated product becomes a peptide comprising C-D-calcitonin CCP mainly exhibiting an action of reducing a serum Ca. In hypothalamic cells, exon of the fifth calcitonin CCP is also spliced

2

and, as a result, protein of the translated product becomes a peptide comprising C-D-CGRP mainly playing a role of regulation of pain and autonomous activity.

When an exon moiety is divided into some as such, it is possible to produce different proteins where several exons are bonded if necessary. Although it has been explained to divide an exon for such a purpose, there has been almost no clarification yet for the necessity of intron except the preparation of an exon moiety. It has been known that many of introns have sequences of 5'-GT and AG-3' at the terminals and that there is an intermediate region abundant in pyrimidine and it has been believed that a splicing is carried out by recognizing those sequences at both terminals.

Phospholipase A<sub>2</sub> is widely distributed in mammals and microbes and it is mostly a membrane-bound enzyme and participates in metabolism of membrane phospholipids. A cytosolic phospholipase A<sub>2</sub> (cPLA<sub>2</sub> $\alpha$ ) of 85 kDa is a kind of phospholipase A<sub>2</sub> and cuts out arachidonic acid mostly from membrane phospholipids producing physiologically active substances by arachidonic acid cascades such as prostaglandin, thromboxane, leukotriene, etc. derived from arachidonic acid. It has been also known that the liberated arachidonic acid participates in various nervous functions in the brain and, until now, the present inventors have shown by a northern blot technique and an in situ hybridization that cPLA<sub>2</sub>  $\alpha$  is abundantly expressed in cranial nerve cells.

On the other hand, kainic acid is a kind of amino acid and has been isolated as an anthelmintic component in *Digenea simplex*. Since kainic acid has a chemical structure similar to glutamic acid, it has been known as a substance binding to a glutamic acid receptor in the brain and the nerve cells of animals resulting in a neuron exciting action.

In order to check the function of phospholipase A<sub>2</sub> in the brain, the present inventors have applied kainic acid stimulation or electric stimulation thereto and found a novel phospholipase A<sub>2</sub> (455 amino acids; molecular weight: about 50 K) which transiently expresses being limited to dentate gyrus of hippocampus. This enzyme is a partial protein initiating from the 308th methionine of a cytosolic phospholipase A<sub>2</sub>a (85 K) and, since it also expresses in genetically defective mouse of the said enzyme, it contains a specific promoter which site-specifically expresses in response to stimulation. Although this enzyme is not present under a non-stimulated state, it is expressed by electric stimulation and kainic acid stimulation and, unlike the conventional phospholipase A<sub>2</sub>, it is independent upon calcium unlike the conventional phospholipase A<sub>2</sub>, produces eicosanoid, regulates a cerebral function and participates in denaturation, apoptosis and regeneration of nerve cells whereby it is believed to be a molecule holding the key to those cerebral functions.

Further, this novel phospholipase A<sub>2</sub> (455 amino acids; molecular weight: about 50 K) is a partial protein initiating from the 308th methionine of a known cytosolic phospholipase A<sub>2</sub>a (85 K), a promoter region specific for expressing this protein is present in the intron moiety immediately before that and the present inventors have found that, in the intron, there is an intron having a function of making the initiation of transcription of RNA possible. Under a usual state, this intron has no function of initiating the transcription of RNA. However, when a certain condition is set, it has a function of initiating the transcription of RNA not from the inherent transcription position but from the moiety of the base sequence of this intron.



## DISCLOSURE OF THE INVENTION

The present invention provides a calcium-independent novel phospholipase A2 (455 amino acids; molecular weight: about 50 K), gene coding therefor and antibody

against that. The present invention further provides an intrinsic promoter or regulatory gene comprising a base sequence existing in intron and site-specifically expressing in response to external stimulation. The present invention furthermore provides a method for expressing a desired protein in response to an external stimulation and to a living thing into which that is introduced.

The present invention still further provides a method for specifically investigating the nerve stem cells since the KIDS cPLA2 of the present invention is specifically expressed in nerve stem cells.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a picture (as a substitute for a drawing) showing the result of a northern blotting using various sites of cPLA2 as probes. The upper column of FIG. 1 shows a base sequence of cPLA2. A, B, C and D show the probes. The middle column of FIG. 1 is the case where no kainic acid treatment is carried out (KA(-)) and the lower column of FIG. 1 is the case of after 3 hours from the kainic acid treatment (KA(+), 3h).

FIG. 2 is a picture (as a substitute for a drawing) showing the result where hippocampus and cerebellum were subjected to a northern blotting with a lapse of time. The left side of FIG. 2 is hippocampus while the right side thereof is cerebellum. In each of them are shown the blots after 0 hour, 0.5 hour, 3 hours, 8 hours, 14 hours and 18 hours from the kainic acid treatment.

FIG. 3 is a picture (as a substitute for a drawing) showing the result of an in situ hybridization in the brain of rat. The left side of FIG. 3 is the result of a cross section of the brain while the right side thereof is that from a vertical section of the brain. In FIG. 3, the parts which are in black are colored parts.

FIG. 4 is a picture (as a substitute for a drawing) where the part of dentate gyrus of hippocampus in the result of FIG. 3 is enlarged. The left side of FIG. 4 is the result where no kainic acid treatment was carried out while the right side thereof is that after 3 hours from the kainic acid treatment.

FIG. 5 is a picture (as a substitute for a drawing) showing the result of confirmation of expression of the desired protein by an immunohistochemical analysis using an antibody specifically recognizing KIDS cPLA2 of the present invention. The left side of the upper column of FIG. 5 is the case where no kainic acid treatment was carried out while the right side thereof is the case of 3 hours after the kainic acid treatment. The left side of the lower column of FIG. 5 is the control where no treatment with an anti-KIDS cPLA2 antibody (IgG) was carried out. The right side of the lower column of FIG. 5 is the result of chromatography in the absence of an anti-KIDS cPLA2 antibody (IgG) after 3 hours from the kainic acid treatment ((-) at the left side of the right side, lower column, FIG. 5) and in the presence of that ((+) at the right side thereof).

FIG. 6 is a picture (as a substitute for a drawing) showing the result of investigation of expression after integration of cDNA coding for cPLA2 $\alpha$  and KIDS cPLA2 of the present invention with an expression vector pTracerEF. In FIG. 6, the lane 1 is the case of a control vector, the lane 2 is the case of cPLA2 $\alpha$ /pTracerEF and the lane 3 is the case of KIDS

cPLA2/pTracerEF. In FIG. 6, the left side is the case where an anti-V5 epitope IgG was used, the middle is the case where an anti-cPLA2 $\alpha$  IgY was used and the right side is the case where an anti-KIDS cPLA2 IgY was used.

FIG. 7 shows the result of enzymatic activity of KIDS cPLA2 and cPLA2 $\alpha$  of the present invention. In FIG. 7, the left side is that for KIDS cPLA2 of the present invention while the right side is that for cPLA2 $\alpha$ . There were used 1-Pam-2-[<sup>14</sup>Cys]arachidonoyl-PC (black dots (●)), 1-Pam-2-[<sup>14</sup>Cys]linoleoyl-PC (black triangles (▲)), 1-Pam-2-[<sup>14</sup>Cys]oleoyl-PC (black squares (■)) and 1-Pam-2-[<sup>14</sup>Cys]palmitoyl-PC (asterisks (\*)) as the substrates.

FIG. 8 shows the result of the test of calcium dependency of KIDS cPLA2 and cPLA2 $\alpha$  of the present invention on enzymatic activity using 1-Pam-2-[<sup>14</sup>Cys]arachidonoyl-PC as a substrate. The solid lines in FIG. 8 are the case of KIDS cPLA2 of the present invention while the broken lines therein are the case of cPLA2 $\alpha$ . In each of them, the black dots (●) are the data in the absence of EDTA Ca while the open circles (○) are those in the presence of EDTA-Ca.

FIG. 9 shows the result given in the above FIG. 8 in terms of a relative ratio.

FIG. 10 is a picture (as a substitute for a drawing) showing the result of investigation of expression of KIDS cPLA2 of the present invention in a mouse defective of cPLA. In FIG. 10, the upper column is that for (+/+) of a knockout mouse while the lower column is that for (-/-) of a knockout mouse. In FIG. 10, the left side is that where no kainic acid treatment was carried out (KA(-)) while the right side is that after 3 hours from the kainic acid treatment (KA (+)).

FIG. 11 illustrates the state of expression of cPLA2 and KIDS cPLA2. In FIG. 11, the upper column schematically shows exon and intron of cPLA2 in genomic gene.

FIG. 12 shows a numbering for a base sequences from the first base of intron immediately before exon containing "Met-308" of rat (upper column), mouse (middle column) and human being (lower column) in which the base wherefrom an exon region of the full-length cPLA2 starts is named 1. The rat sequence is represented by SEQ ID NO: 13 (nucleotides 1 to 630 of SEQ ID NO: 13 correspond to rat nucleotides -630 to -1 of FIG. 12) and SEQ ID NO: 6 (nucleotides 1 to 130 of SEQ ID NO: 6 correspond to rat nucleotides +1 to +130 of FIG. 12). The mouse sequence is represented by SEQ ID NO: 14 (nucleotides 1 to 739 of SEQ ID NO: 14 correspond to mouse nucleotides -638 to +101) and SEQ ID NO: 9 (nucleotides 107 to 133 of SEQ ID NO: 9 correspond to mouse nucleotides +102 to +128). The human being sequence is represented by SEQ ID NO: 15 (nucleotides 1 to 207 of SEQ ID NO: 15 correspond to human nucleotides -673 to -467 of FIG. 12), SEQ ID NO: 12 (nucleotides 1 to 560 of SEQ ID NO: 12 correspond to nucleotides -466 to +94 of FIG. 12), and SEQ ID NO: 2 (nucleotides 96 to 122 of SEQ ID NO: 2 correspond to nucleotides +95 to +121).

FIG. 13 is a picture (as a substitute for a drawing) showing the result of investigation of expression of KIDS cPLA 2 of the present invention using nerve stem cells and mature nerve cells. In FIG. 13, the upper column is nestin as a control, the middle column is the case where nerve stem cells were used and the lower column is the case where mature cells of nerve were used. Pictures on the left side (A) show the positions of each of the cells, pictures on the middle (B) are coloration showing the expression of KIDS cPLA 2 of the present invention and pictures at the right side are those where A at the left side and B at the middle were piled to confirm the positions in both.



## 5

FIG. 14 is a picture (as a substitute for a drawing) showing the result of investigation of expression of KIDS cPLA 2 of the present invention using nerve stem cells by means of stimulation with kainic acid, with kainic acid and CNQX and with glutamic acid. In FIG. 14, the probe used is P90-P27 of 252 bp in the upper column, P19-P27 of 290 bp in the second column and G3 PDH and nestin in the lower two columns as controls. The lowermost picture of FIG. 14 shows the initiation positions for transcription of KIDS cPLA 2 at the 5'-side and sequential positions of the probes used in the upper two columns in FIG. 14. The lanes in FIG. 14 are control and stimulations by kainic acid (KA(10  $\mu$ M)), by kainic acid and CNQX (KA(10  $\mu$ M)+CNQX (20  $\mu$ M)) and by glutamic acid (Glu (50  $\mu$ M)) from the left side.

BEST MODE FOR CARRYING OUT THE  
INVENTION

The present invention relates to a calcium-independent novel phospholipase A2 and, more particularly, it relates to a calcium-independent and hippocampus-specific phospholipase A2 which is a phospholipase A2 having an amino acid sequence described in SEQ ID NO: 1, NO: 5 or NO: 8 of the Sequence Listing or an amino acid sequence where one or more amino acid(s) in the amino acid sequence is/are substituted with other amino acid(s) or deficient or one or more amino acid(s) is/are added thereto, to gene coding therefor and an antibody where full length or fragment thereof is an antigen.

The present further relates to gene having a base sequence existing in intron where the base sequence is able to make the initiation of transcription of RNA by external stimulation such as kainic acid stimulation or electric stimulation possible and, more particularly, it relates to gene which is able to make the initiation of transcription of RNA possible in a site-specific manner. Preferred examples of the gene of the present invention are genes having the base sequence described in SEQ ID NO: 12, NO: 13 or NO: 14 of the Sequence Listing and having the base sequence comprising a partial sequence where a part thereof is deleted, added or substituted.

The present invention further relates to a promoter having a base sequence existing in intron and being able to make the initiation of transcription of RNA by external stimulation such as kainic acid stimulation or electric stimulation possible and, more particularly, it relates to the above-mentioned promoter where the initiation of transcription of RNA is in a site-specific manner and to a regulatory gene having a regulatory element at the upper stream of the said promoter.

The present invention furthermore relates to a process wherein any of the above-mentioned gene, the above-mentioned promoter or the above-mentioned regulatory gene is introduced into the upper stream of the gene coding for protein to initiate the transcription of RNA by external stimulation such as kainic acid stimulation or electric stimulation preferably in a site-specific manner whereby the said protein is expressed in response to the external stimulation and to a living thing wherein any of the above-mentioned gene, the above-mentioned promoter and the above-mentioned regulatory gene is introduced into the upper stream of the gene coding for protein.

The present invention still further relates to a method for a specific investigation of nerve stem cells by expression of KIDS cPLA 2 of the present invention. Thus, the present invention relates to a method for the detection or the identification of nerve stem cells by detecting or identifying

## 6

the mRNA coding for a calcium-independent and novel phospholipase A2 (to be more specific, a phospholipase A2 which is calcium-independent and hippocampus-specific and has an amino acid sequence described in SEQ ID NO: 1, NO: 5 or NO: 8 of the Sequence Listing or an amino acid sequence where one or more amino acid(s) is/are substituted with other amino acid or deleted or one or more amino acid(s) is/are added) by stimulating the nerve cells by external stimulation.

During a course of the study for investigating the function of phospholipase A2 in the brain, the present inventors prepared slices of the brain of rat into which kainic acid was intraperitoneally injected and histochemically checked the expression of mRNA using cPLA2 as a probe (searching element). For the selection of a probe, confirmation was carried out by means of a northern blotting usually using different sites of the cPLA2 whereupon it was found that, when a specific site (5'-terminal) was used, mRNA having a shorter length (about 1.8 kilo base pairs) than cPLA2 was induced.

Result of the northern blotting is shown in FIG. 1 as a picture which is a substitute for a drawing. The upper column of FIG. 1 shows a base sequence of cPLA2. The left end is a translation initiation codon (ATG) and the parts used as a probe are shown by A, B, C and D. Thus, probe A is a part from BamHI to Ball, probe B is a part from RsaI to RsaIBall, probe C is a part from RsaI to Ball and probe D is a part from RsaI to termination codon (TGA).

The middle column of FIG. 1 is the case where no kainic acid treatment was carried out (KA(-)). The lower column of FIG. 1 is the case after 3 hours from the kainic acid treatment (KA(+), 3h). When no kainic acid treatment was carried out (KA(-)) (the middle column of FIG. 1), a plot was noted at the position of cPLA2 $\alpha$  only while, in the case of 3 hours after a kainic acid treatment when that treatment was carried out (KA(+)), plots of shorter chain length were able to be observed in probes B, C and D at the 5'-terminal side not only at the position of cPLA2 $\alpha$  but also at the position beneath that.

Then, a northern blotting was carried out with a lapse of time for hippocampus and cerebellum. The result is shown by a picture in FIG. 2 as a substitute for a drawing. The left side of FIG. 2 is for hippocampus while the right side thereof is for cerebellum. Each of them shows the blots after 0 hour, 0.5 hour, 3 hours, 8 hours, 14 hours and 18 hours from the kainic acid treatment. At any place of FIG. 2, plot was found at the position of cPLA2 $\alpha$  while, only at the area after 3 hours from the kainic acid treatment in the case of hippocampus (left side of FIG. 2), there were observed plots of shorter chain length not only at the position of cPLA2 $\alpha$  but also beneath that.

When an in situ hybridization was further carried out, a specific expression was noted at dentate gyrus of hippocampus. The result is shown by a picture in FIG. 3 as a substitute for a drawing. The left side of FIG. 3 is the result of a cross section of the brain while the right side thereof is that from a vertical section of the brain. In FIG. 3, the part which is in black is a colored part. The colored part is dentate gyrus of hippocampus.

The result is enlarged and is shown in FIG. 4 which is a picture as a substitute for a drawing. The left side of FIG. 4 is the result where no kainic acid treatment was carried out while the right side thereof is that after 3 hours from the kainic acid treatment. Coloration was able to be observed around the dentate gyrus of hippocampus. This coloration was strong at the outside of the dentate gyrus of hippocampus and, in the dentate gyrus of hippocampus, there are



many nerve stem cells whereby that is presumed to be due to the nerve stem cells existing in the dentate gyrus of hippocampus.

Therefore, the full length of cPLA2 was used as a probe and the desired cDNA was obtained from a library of dentate gyrus of hippocampus. This cDNA was translated into protein and its enzymatic activity was checked whereupon a phospholipase A2 activity was found.

From the structure analysis, that was found to be a phospholipase A2 molecule of a shortened type of phospholipase A2 of a cytoplasmic type (cytosolic phospholipase A2; abbreviated as cPLA2). cDNA of rat was a protein with a molecular weight of 50,810.6 comprising 445 amino acids having 1,842 base pairs where the translated region was 1,355 base pairs. Since this phospholipase A2 of a shortened type is specifically expressed in the dentate gyrus of hippocampus after stimulation with kainic acid, it was named a kainate-inducible dentate gyrus specific cPLA2 (KIDS cPLA2).

Amino acid sequence of the resulting KIDS cPLA2 is shown by way of one-letter code of amino acid as follows.

KIDS cPLA2 of human being is as follows.

```

M N T T L S S L K E K V N T A Q C P L P   2 0
L F T C L H V K P D V S E L M F A D W V   4 0
E F S P Y E I G M A K Y G T F M A P D L   6 0
F G S K F F M G T V V K K Y E E N P L H   8 0
F L M G V W G S A F S I L F N R V L G V   1 0 0
S G S Q S R G S T M E E E L E N I T T K   1 2 0
H I V S N D S S D S D D E S H E P K G T   1 4 0
E N E D A G S D Y Q S D N Q A S W I H R   1 6 0
M I M A L V S D S A L F N T R E G R A G   1 8 0
K V H N F M L G L N L N T S Y P L S P L   2 0 0
S D F A T Q D S F D D D E L D A A V A D   2 2 0
P D E F E R I Y E P L D V K S K K I H V   2 4 0
V D S G L T F N L P Y P L I L R P Q R G   2 6 0
V D L I I S F D F S A R P S D S S P P F   2 8 0
K E L L L A E K W A K M N K L P F P K I   3 0 0
D P Y V F D R E G L K E C Y V F K P K N   3 2 0
P D M B K D C P T I I H F V L A N I N F   3 4 0
R K Y K A P G V P R E T E E E K E I A D   3 6 0
F D I F D D P E S P F S T F N F Q Y P N   3 8 0
Q A F K R L H D L M H F N T L N N I D V   4 0 0
I K E A M V E S I E Y R R Q N P S R C S   4 2 0
V S L S N V E A R R F F N K E F L S K P   4 4 0
K A 4 4 2
    
```

KIDS cPLA2 of rat is as follows.

```

M S T T L S S L K E K V S A A R G P L P   2 0
L F T C L H V K P D V S E L M F A D W V   4 0
    
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-continued

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E F S P Y E I G M A K Y G T F M T P D L   6 0
5 F G S K F F M G T V V K K Y E E N P L H   8 0
F L M G V W G S A F S I L F N R V L G V   1 0 0
S G S Q N K G S T M E E E L E N I T A K   1 2 0
10 H I V S N D S S D S D D E A Q G P K G T   1 4 0
E N E D A E R E Y Q N D N Q A S W V H R   1 6 0
M L M A L V S D S A L F N T R B G R A G   1 8 0
15 K E H N F M L G L N L N T S Y P L S P L   2 0 0
R D F S P Q D S F D D D E L D A A V A D   2 2 0
P D E F E R I Y E P L D V K S K K I H V   2 4 0
V D S G L T F N L P Y P L I L R P Q R G   2 6 0
20 V D L I I S F D F S A R P S D T S P P F   2 8 0
K E L L L A E K W A K M N K L P F P K I   3 0 0
25 D P Y V F D R E G L K E C Y V F K P K N   3 2 0
P D V E K D C P T I I H F V L A N I N F   3 4 0
R K Y K A P G V L R E T K E E K E I A D   3 6 0
F D I F D D P E S P F S T F N F Q Y P N   3 8 0
30 Q A F K R L H D L M Y F N T L N N I D V   4 0 0
I K D A I V E S I E Y R R Q N P S R C S   4 2 0
V S L S N V E A R K F F N K E F L S K P   4 4 0
35 T A E S I 4 4 5
    
```

KIDS cPLA2 of mouse is as follows.

```

40 M S M T L S S L K E K V N A A R C P L P   2 0
L F T C L H V K P D V S E L M F A D W   4 0
V E F S P Y E I G M A K Y G T F M A P D   6 0
45 L F G S K F F M G T V V K K Y E E N P L   8 0
H F L M G V W G S A F S I L F N R V L G   1 0 0
V S G S Q N K G S T M E E E L E N I T A   1 2 0
50 K H I V S N D S S D S D D E A Q G P K G   1 4 0
T E N E E A E K E Y Q S D N Q A S W V H   1 6 0
R M L M A L V S D S A L F N T R E G R A   1 8 0
55 G K V H N F M L G L N L N T S Y P L S P   2 0 0
L R D F S S Q D S F D D E L D A A V A D   2 2 0
P D E F E R I Y E P L D V K S K K I H V   2 4 0
V D S G L T F N L P Y P L I L R P Q R G   2 6 0
60 V D L I I S F D F S A R P S D T S P P F   2 8 0
K E L L L A E K W A K M N K L P F P K I   3 0 0
D P Y V F D R E G L K E C Y V F K P K N   3 2 0
65 P D V E K D C P T I I H F V L A N I N F   3 4 0
    
```



-continued

R K Y K A P G V L R E T K E E K E I A D 3 6 0  
 E D I F D D P E S P F S T F N F Q Y P N 3 8 0  
 Q A F K R L H D L M Y F N T L N N I D V 4 0 0  
 I K D A I V E S I E Y R R Q N P S R C S 4 2 0  
 V S L S N V E A R K F F N K E F L S K P 4 4 0  
 T V 4 4 1

Amino acid sequence of KIDS cPLA2 of human being is shown in SEQ ID NO: 1 of the Sequence Listing. Base sequence of translated region of cDNA of KIDS cPLA2 of human being is shown in SEQ ID NO: 2, NO: 3 and NO: 4 of the Sequence Listing. SEQ ID NO: 2 is that where the sequence of 5' UTR is made type I, SEQ ID NO: 3 is that where the sequence of 5' UTR is made type II and SEQ ID NO: 4 is that where the sequence of 5' UTR is not classified into type I and type II.

Amino acid sequence of KIDS cPLA2 of rat is shown in SEQ ID NO: 5 of the Sequence Listing. Base sequence of the translated region of cDNA of KIDS cPLA2 of rat is shown in SEQ ID NO: 6 and NO: 7 of the Sequence Listing. SEQ ID NO: 6 is that for type I and SEQ ID NO: 7 is that for type II.

Amino acid sequence of KIDS cPLA2 of mouse is shown in SEQ ID NO: 8 of the Sequence Listing. Base sequence of the translated region of cDNA of KIDS cPLA2 of mouse is shown in SEQ ID NO: 9, NO: 10 and NO: 11 of the Sequence Listing. SEQ ID NO: 9 is that where the sequence of 5' UTR is made type I, ID SEQ NO: 10 is that where the sequence of 5' UTR is made type II and SEQ ID NO: 11 is that where the sequence of 5' UTR is not classified into type I and type II.

A polyclone antibody (stump antibody) which specifically recognizes the KIDS cPLA2 of the present invention was prepared. Expression of the desired protein was confirmed by immunohistochemical analysis using that antibody. The result is shown in FIG. 5 which is a picture as a substitute for a drawing. The left side of the upper column of FIG. 5 is the case where no kainic acid treatment was carried out while the right side thereof is the case of 3 hours after the kainic acid treatment. Colored parts due to the antibody can be observed. The left side of the lower column of FIG. 5 is the control where no treatment with an anti-KIDS cPLA2 antibody (IgG) was carried out. The right side of the lower column of FIG. 5 is the result of chromatography in the absence of an anti-KIDS cPLA2 antibody (IgG) after 3 hours from the kainic acid treatment ((-) at the left side of the right side, lower column, FIG. 5) and in the presence of that ((+) at the right side thereof).

Then, cDNA coding for KIDS cPLA2 of the present invention and cPLA2 was integrated with an expression vector pTracer EF and its expression was investigated. The result is shown in FIG. 6 which is a picture as a substitute for a drawing. In FIG. 6, the lane 1 is the case of a control vector, the lane 2 is the case of cPLA2 $\alpha$ /pTracer EF and the lane 3 is the case of KIDS cPLA2/pTracer EF. In FIG. 6, the left side is the case where an anti-V5 epitope IgG was used, the middle is the case where an anti-cPLA2 $\alpha$  IgY was used and the right side is the case where an anti-KIDS cPLA2 IgG was used.

Each spot by the anti-V5 epitope IgG and spot of KIDS cPLA2 by the anti-KIDS cPLA2 IgG were confirmed whereby expression of KIDS cPLA2 was confirmed.

Then enzymatic activity of cPLA2 $\alpha$  and KIDS cPLA2 of the present invention were investigated. There were used 1-Pam-2-[<sup>14</sup>C]arachidonoyl-PC (black dots (●) in FIG. 7), 1-Pam-2-[<sup>14</sup>C]linoleoyl-PC (black triangles (▲) in FIG. 7), 1-Pam-2-[<sup>14</sup>C]oleoyl-PC (black squares (■) in FIG. 7) and 1-Pam-2-[<sup>14</sup>C]palmitoyl-PC (asterisks (\*) in FIG. 7) as the substrates for testing the enzymatic activity of each of them. The result is shown in FIG. 7. In FIG. 7, the left side is that for KIDS cPLA2 of the present invention while the right side is that for cPLA2 $\alpha$ . Each and any of the enzymes showed very high enzymatic activity to arachidonic acid phospholipids and was found to have nearly the same activity as phospholipase A2.

Values of those enzymatic activities (pmol/minute) are shown in the following Table 1.

TABLE 1

Enzymatic Activities of KIDS cPLA2 and cPLA2 $\alpha$ 

Substrate	Phospholipase A2 Activity (pmol/min)	
	KIDS cPLA2	cPLA2 $\alpha$
1-Pam-2-[ <sup>14</sup> C]arachidonoyl-PC	35.6 $\pm$ 3.8	24.4 $\pm$ 1.4
1-Pam-2-[ <sup>14</sup> C]linoleoyl-PC	20.1 $\pm$ 1.7	11.9 $\pm$ 1.8
1-Pam-2-[ <sup>14</sup> C]oleoyl-PC	14.3 $\pm$ 1.5	9.1 $\pm$ 1.1
1-Pam-2-[ <sup>14</sup> C]palmitoyl-PC	9.4 $\pm$ 1.0	9.8 $\pm$ 1.7

Incidentally, the phospholipase A2 activity is given in terms of the difference from the control.

Then, calcium dependency of cPLA2 $\alpha$  and KIDS cPLA2 of the present invention on enzymatic activity was investigated using 1-Pam-2-[<sup>14</sup>C]arachidonoyl-PC as a substrate. EDTA-Ca was used as a calcium source.

The result is shown in FIG. 8 and FIG. 9. The solid lines in FIG. 8 are the case of KIDS cPLA of the present invention while the broken lines therein are the case of cPLA2 $\alpha$ . In each of them, the black dots (●) are the data in the absence of EDTA-Ca while the open circles (○) are those in the presence of EDTA-Ca. It is noted that, in the case of cPLA2 $\alpha$ , there is a sudden reduction in the activity by the presence of calcium while, in the case of KIDS cPLA2 of the present invention, there is no such a reduction in the activity.

FIG. 9 shows the above-mentioned result in terms of a relative ratio. It is noted that, in the case of KIDS cPLA2 of the present invention, an activity of around 40% is maintained even in the presence of calcium while, in the case of cPLA2 $\alpha$ , the activity is reduced to an extent of around 10-15% in the presence of calcium.

As such, KIDS cPLA2 of the present invention is characteristic in being calcium-independent as compared with the conventional cPLA2 $\alpha$ .

Then expression of KIDS cPLA2 of the present invention in cPLA-defective mouse prepared by Shimizu, et al. (Uozumi, N. et al., *Nature*, 390, 618-622, 1997) was investigated. The result is shown in FIG. 10 which is a picture as a substitute for a drawing. In FIG. 10, the upper column is that for (+/+) of a knockout mouse while the lower column is that for (-/-) of a knockout mouse. In FIG. 10, the left side is that where no kainic acid treatment was carried out (KA (-)) while the right side is that after 3 hours from the kainic acid treatment (KA (+)). In any of the knockout mice, expression of the present enzyme was able to be confirmed by a kainic acid treatment.



## 11

The above shows that KIDS cPLA2 of the present invention expresses using a promoter which is different from its full-length cPLA2.

FIG. 11 illustrates the state of expression of cPLA2 and KIDS cPLA2. In FIG. 11, the upper column schematically shows exon and intron of cPLA2 in genomic gene. The full-length cPLA2 is produced from all exons and regulatory gene containing a promoter region is present in the upper stream of the initial exon. On the contrary, KIDS cPLA2 of the present invention is a protein starting from the 308th methionine mentioned as "M-308" in FIG. 11 and, since expression of this protein was confirmed in the cPLA-defective mouse, i.e., a mouse where the function of regulatory gene containing a promoter region in the upper stream of the initial exon, it has been found that KIDS cPLA2 of the present invention has a regulatory gene region containing a promoter region in the upper stream of "M-308". However, the said regulatory gene is in such a manner that, under an ordinary state, the gene does not function and has also been noted to function only by the stimulation such as by a kainic acid stimulation.

Therefore, a base sequence of upper stream of "M-308" was analyzed for rat, mouse and human being. The result is aligned and shown in FIG. 12.

The base sequence of this intron in human being is shown in SEQ ID NO: 12 of the Sequence Listing. The base sequence thereof in rat is shown in SEQ ID NO: 13 of the Sequence Listing. Further, the base sequence thereof in mouse is shown in SEQ ID NO: 14 of the Sequence Listing.

FIG. 12 shows a numbering for base sequences from the first base of intron immediately before exon containing "M-308" of rat (upper column), mouse (middle column) and human being (lower column) in which the base wherefrom an exon region of the full-length cPLA2 starts is named No.1. ATG from the 92nd one (human being) in this numbering is a translation initiation codon of KIDS cPLA2.

Then, expression of KIDS cPLA 2 of the present invention was investigated using nerve cells in the dentate gyrus of hippocampus of the brain. The result is shown in FIG. 13 which is a picture as a substitute for a drawing.

In FIG. 13, the upper column is nestin as a control, the middle column is the case where nerve stem cells were used and the lower column is the case where mature cells of nerve were used. A in the left side shows the positions of each of the cells, B in the middle is coloration showing the expression of KIDS cPLA 2 of the present invention and the right side is that where A at the left side and B at the middle were piled to confirm the positions in both.

As the result, it is noted that no clear expression is observed for KIDS cPLA2 of the present invention in nerve mature cells but a clear expression is observed in nerve stem cells. The above suggests that KIDS cPLA2 of the present invention is a substance which is specifically expressed in nerve stem cells and that, in nerve stem cells, intron in mature cells specifically plays a role of a promoter.

Then expression of KIDS cPLA 2 of the present invention using nerve stem cells by means of stimulation by kainic acid (10  $\mu$ M), by kainic acid and CNQX (10  $\mu$ M KA and 20  $\mu$ M CNQX) and by glutamic acid (50  $\mu$ M) was investigated.

The result is shown in FIG. 14 which is a picture as a substitute for a drawing. In FIG. 14, the probe used is P90-P27 of 252 bp (the said sequence is a sequence of a moiety which is common in the full-length cPLA2) in the upper column, P19-P27 of 290 bp (the said sequence contains a sequence specific to KIDS cPLA2 of the present invention) in the second column and G3 PDH and nestin in the lower two columns as controls. The lowermost picture of

## 12

FIG. 14 shows the initiation positions for transcription of KIDS cPLA 2 at the 5'-side and sequential positions of the probes used in the upper two columns in FIG. 14.

The lanes in FIG. 14 are control and stimulations by kainic acid (KA (10  $\mu$ M)), by kainic acid and CNQX (KA (10  $\mu$ M)+CNQX (20  $\mu$ M)) and by glutamic acid (Glu (50  $\mu$ M)) from the left side.

As a result, in the case of stimulation by kainic acid (10  $\mu$ M), a specific expression of KIDS cPLA2 of the present invention was confirmed.

Accordingly, the present invention provides a method for searching the nerve stem cells in a specific manner by expression of KIDS cPLA2 of the present invention. Thus, according to such a method of the present invention, cells which are to be the candidates are stimulated by kainic acid and expression of KIDS cPLA2 of the present invention is observed whereby the nerve stem cells are able to be specifically and easily searched.

The KIDS cPLA2 of the present invention is in a partial length of the full-length cPLA2. It is characterized in maintaining a phospholipase A2 activity and having a calcium-independent property and is not always limited to that having an amino acid sequence described in SEQ ID NO: 1, NO: 5 or NO: 8 of the Sequence Listing. So far as it maintains the phospholipase A2 activity and is calcium-independent, about 1-200 or preferably about 1-100, 1-50 or 1-20 amino acid(s) described in SEQ ID NO: 1, NO: 5 or NO: 8 of the Sequence Listing may be substituted with other amino acid(s) or may be deleted therefrom or added thereto. It is also possible that such substitution, deletion and addition may be simultaneously carried out in a combined manner.

Although the KIDS cPLA2 of the present invention may be manufactured according to a process disclosed in the present specification, it may also be manufactured by a conventional gene recombination technique using cDNA of the KIDS cPLA2 of the present invention.

When a full length or a part of the KIDS cPLA2 of the present invention or, preferably, a peptide comprising 10 or more amino acids is used as an antigen, it is possible to manufacture an antibody thereto. The antibody of the present invention may be manufactured by a conventional process and, if necessary, it is possible to manufacture a polyclonal antibody or a monoclonal antibody.

It has been also known that cell death specific to the dentate gyrus of hippocampus takes place by a kainic acid stimulation, by a fit of epilepsy, etc. The present inventors have found that KIDS cPLA2 is expressed in the dentate gyrus of hippocampus by a kainic acid stimulation, by a fit of epilepsy, etc. In view of the above, cell death in the dentate gyrus of hippocampus can be prevented by preparing an inhibitor for the said enzyme and the said enzyme is useful for a development of such an inhibitor as well.

Further, the present inventors have found for the first time that some of intron has a function as a regulatory gene which is activated by an external stimulation and have clarified that at least a base sequence which also functions as a promoter responding to the external stimulation is present in the base sequence of intron.

Accordingly, the present invention provides a gene having a base sequence existing in intron where the said base sequence is able to make the initiation of transcription of RNA by external stimulation possible. The said gene of the present invention comprises at least six bases. Preferably, it is an oligonucleotide having a base sequence existing in intron and comprising at least four or, preferably, at least six bases in the base sequence shown in SEQ ID NO: 12, NO:



13 or NO: 14 of the Sequence Listing where the said base sequence is able to make the initiation of transcription of RNA by external stimulation possible.

Since the said gene of the present invention has a function at least as a promoter participating in initiation of transcription of RNA, the present invention also provides a promoter comprising the said gene or its partial length and being able to make the initiation of transcription of RNA by external stimulation possible. The promoter of the present invention is characterized in that it does not generate the initiation of transcription of RNA under an ordinary condition of mature cells but is able to generate the initiation of transcription of RNA only by a specific external stimulation. The promoter of the present invention is also characterized in that its base sequence is a base sequence existing in intron. More preferably, it is characterized in that the site whereby the initiation of transcription of RNA is made possible is specific. It is preferred that the promoter of the present invention has a length of at least 4-20 bases or, preferably, at least 6-20 bases although the present invention is not limited thereto.

Although the promoter of the present invention may be used solely, it is preferred to use it together with a regulatory element such as an enhancer. Although the regulatory enhancer is positioned at cis, it may be at trans as well. The present invention provides a regulatory gene in which the regulatory element and the promoter of the present invention as mentioned above are in a set. When the regulatory element is a cis-element, such a regulatory gene may be in single-stranded or may be in double-stranded. It is used as double strands when the regulatory element is a trans-element.

When an intron is known to have a base sequence which makes the initiation of transcription of RNA by external stimulation possible while it is not well known that which base sequence in the intron plays a role as a promoter, etc., then the full length of the said intron may be used as a regulatory gene of the present invention.

The term "external stimulation" used in the present invention is a stimulation which does not take place under the growth condition of ordinary mature cells and, preferably, it is a stimulation by which cell death is induced. Thus, there may be exemplified stimulation by a chemical substance such as kainic acid; physical stimulation such as electric shock, temperature change, etc.; stimulation by disorder of other organs such as a fit of epilepsy; and the like.

The term "site-specific" used in the present invention means that a thing is specific to a site which is able to be discriminated from others in terms of type, state, growth degree, etc. of tissues, organs or cells in living body. Although the promoter, gene, etc. of the present invention which are able to make the initiation of transcription of RNA by external stimulation possible may not be always site-specific, they may be site-specific as well. The base sequence of intron shown by SEQ ID NO: 12, NO: 13 and NO: 14 of the Sequence Listing of the present specification is believed to be specific to the dentate gyrus of hippocampus although the promoter, gene, etc. of the present invention is not limited thereto.

The present invention is to clarify the presence of a base sequence which makes the initiation of transcription of RNA by external stimulation in intron of living thing possible and the range of utilization of such a gene of the present invention is quite broad. The first characteristic is that, since it is present as intron, even when the gene is introduced, it usually functions only as intron and does not affect the ordinary growth of living things. The second characteristic

is that the regulatory gene of the present invention is inactive for the transcription of RNA under an ordinary state and does not express the protein coded at its downstream. The third characteristic is that it is also possible to make it expressed in a site-specific manner.

Since the promoter and regulatory gene of the present invention have such characteristics, applications according to the particular object are possible. For example, when it is an object that a partial length of a protein is expressed whereby its physiological activity is observed in vivo, the gene of the present invention is introduced immediately before the exon containing methionine which is to be an initiation codon to give an external stimulation to living body whereby expression of protein having a desired partial length can be promoted. When there is no suitable methionine, it may be also possible to introduce a base sequence coding for methionine into an intron region.

According to the second characteristic, gene bonded with the regulatory gene and promoter of the present invention at the upper stream of the desired protein is introduced into living body to give a specific external stimulation whereby the expression of the desired introduced protein is expressed only at the stage of giving the external stimulation. For example, it is possible that a physiologically active protein is added to the end of the promoter of the present invention and, only when a specific external stimulation is applied, the said physiologically active protein is expressed and the said physiological activity is temporarily given to the cell. When a toxin such as diphtheria toxin is used as a physiologically active protein, it is now possible to kill the cells in a transient manner. Alternatively, the gene to which CRE gene is connected to the downstream of the promoter of the present invention is introduced whereupon there is prepared a transgenic mouse where specific gene such as glutamic acid receptor is surrounded by a lox-P sequence. By doing so, CRE gene is expressed and specific gene such as glutamic acid receptor surrounded by a lox-P sequence is deleted by a homologous recombination when a specific external stimulation is applied and, therefore, it is possible to prepare a mouse deficient in specific gene such as a glutamic acid receptor as from the stage of application of a specific external stimulation. It is now possible by such a transgenic mouse to precisely analyze the pathology in mature living body where specific gene such as a glutamic acid receptor is deficient.

Further, according to the above-mentioned third characteristic, it is possible to bring about the above-mentioned characteristic in a site-specific manner in a living body. For example, it is possible to destroy a specific gene specifically in the dentate gyrus of hippocampus.

Accordingly, the present invention provides a method where expression of gene coding for a protein introduced into a living body using the promoter and regulatory gene of the present invention is regulated by a specific external stimulation. As mentioned already, it is possible according to this method of the present invention that expression length, expression time and expression site of the introduced protein are regulated.

There is no particular limitation as to the protein which is introduced in this method of the present invention provided that it is a protein having any physiological activity and the protein can be introduced in a state of genome or in a state of cDNA. The protein having a physiological activity may be, for example, that which has the so-called physiological activity such as hormones and cytokines, toxin such as diphtheria toxin or that which induces a homologous recombination such as CRE gene.



## 15

The present invention also provides a living thing into which the promoter and regulatory gene of the present invention are introduced at the upper stream of the gene coding for protein. The living thing of the present invention is useful as an experimental animal and is applicable, for example, to mouse, rat, rabbit and monkey. It is also possible to apply to plants.

With regard to such experimental animals, there have been developed transgenic mouse, knockout mouse, etc. In a knockout mouse, there has been a demand for development of a conditional targeting method and, as being noted from the fact that a tissue-specifically expressing promoter, a tetracycline-sensitive promoter, etc. have been developed, there has been a demand for development of a promoter which is tissue-specific and stage-specific. The promoter and regulatory gene of the present invention satisfy such requirements and also have a function as intron and, therefore, the promoter and regulatory gene of the present invention can be widely applied to experimental animals.

## EXAMPLES

Now the present invention will be illustrated in more detail by way of the following Examples although the present invention is not limited to those Examples only.

## Example 1

## Northern Blotting Using Various Probes of cPLA2

cDNA of cPLA2 $\alpha$  of rat was divided into four main region, i.e., A, B, C and D from the 5'-terminal. Length of each region was made around 300-500 bp and, after such a cDNA fragment was integrated with a riboprobe synthetic vector, a radio-labeled riboprobe was synthesized by an in vitro transcription method. A hybridization reaction was carried out using a membrane which was blotted with poly(A)<sup>+</sup> RNA of hippocampal dentate gyrus and hippocampus of rat subjected to a kainic acid stimulation and a riboprobe of each of A, B, C and D to check which probe was able to detect the KIDS cPLA2. As a result, it was found that KIDS cPLA2 mRNA was detected in riboprobes of B, C and D except A.

The result is shown in FIG. 1 and FIG. 2.

## Example 2

## In Situ Hybridization

Brain of Wister rat of 3 weeks age stimulated by kainic acid was fixed by 4% paraformaldehyde and then a frozen slice was prepared. Each of the radio-labeled riboprobes B, C and D was subjected to a hybridization reaction with the frozen slice and the labeled image of the slice from each probe was confirmed to be same. Then, frozen slice of the brain was prepared again using riboprobe C with or without a kainic acid stimulation and an expression pattern of KIDS cPLA2 mRNA was investigated. As a result, KIDS cPLA2 mRNA was found to be drastically induced in the hippocampal dentate gyrus. When a strongly enlarged image was observed under a microscope, KIDS cPLA2 mRNA was found to express particularly abundantly in the innermost layer of the dentate gyrus.

The result is shown in FIG. 3 and FIG. 4.

## 16

## Example 3

## Immunohistochemical Dyeing

Brains of cPLA2a knockout mouse and C57/Black 6J mouse of 6-10 weeks age and Wister rat of 3 weeks age stimulated by kainic acid were fixed with 4% paraformaldehyde and then frozen slices were prepared. Immunoreaction of an anti-KIDS cPLA2-specific stump antibody with the brain slice was carried out overnight at 4° C. and expression of KIDS cPLA2 protein was confirmed by a secondary antibody labeled with gold colloid. As a result, it was found that, like mRNA, KIDS cPLA2 was drastically induced in the hippocampal dentate gyrus and further that such an expression was noted in the hippocampal dentate gyrus of cPLA2a knockout mouse as well. From the above, it was suggested that a promoter of KIDS cPLA2 was present at the downstream of the eighth exon of cPLA2a destroyed by a cPLA2a knockout mouse and found that an isoform of cPLA2a was induced by acute nervous stimulation.

The result is shown in FIG. 5.

## Example 4

## Cloning of cDNA of KIDS cPLA2 of Rat

Clone was isolated after confirming its presence by two kinds of method.

(1) A cDNA library was prepared using poly(A)<sup>+</sup> RNA purified from hippocampus of rat after stimulation by kainic acid and a positive clone was selected using a cDNA sequence of 1,365 (Rsa I)-1,925 (Bal I) from the initiation point for translation of cPLA2a which is able to detect KIDS cPLA2 as a probe.

From 4,000,000 clones were selected 12 positive clones. Among them, two are those of full-length phospholipase A2 while six and four among the residual ten were different in their type. The former was named type II and the latter was named type I.

(2) In order to confirm the 5'-terminal of KIDS cPLA2 cDNA of rat, a 5' RACE method (5'-rapid amplification of cDNA ends) was carried out using poly(A)<sup>+</sup> RNA purified from the hippocampus of rat after stimulation by kainic acid. A sequence amplified to the 5'-upper stream from the primer existing in the above-mentioned sequence of 1,365 (Rsa I)-1,925 (Bal I) was identical with a sequence of clone selected from the cDNA library.

From the above, KIDS cPLA2 was found to be a novel gene induced in hippocampus after stimulation by kainic acid.

An amino acid sequence of the resulting KIDS cPLA2 of rat is shown in SEQ ID NO: 5 of the Sequence Listing. Base sequences of translated region of cDNA of KIDS cPLA2 of rat are shown in SEQ ID NO: 6 (type I) and SEQ ID NO: 7 (type II) of the Sequence Listing.

An amino acid sequence of KIDS cPLA2 of mouse is shown in SEQ ID NO: 8 of the Sequence Listing. Base sequences of translated region of KIDS cPLA2 of mouse are shown in SEQ ID NO: 9 (in the case that 5'UTR is defined as type I), in SEQ ID NO: 10 (in the case that 5'UTR is defined as type II) and in SEQ ID NO: 11 (in the case that 5'UTR is not divided into types I and II), respectively, of the Sequence Listing.

An amino acid sequence of KIDS cPLA2 of human being is shown in SEQ ID NO: 1 of the Sequence Listing. Base



sequences of translated region of cDNA of KIDS cPLA2 of human being are shown in SEQ ID NO: 2 (when 5'UTR was made type I), SEQ ID NO: 3 (when 5'UTR was made type II) and SEQ ID NO: 4 (when 5'UTR was not divided into types I and II) of the Sequence Listing.

Those sequences are selected from the most appropriate sequences after the cDNA sequences of human being, mouse and rat were subjected to an alignment program at the same time and then applied with the conditions such as the position of initiation of transcription, the position of nucleotide presumed to be the transcription initiation position for each of type I and type II, the junction sequence connecting the sequence of type I and the sequence of type II, the sequence homology as a whole, etc.

#### Example 7

##### Manufacture of an Antibody of KIDS cPLA2

In order to specifically detect the KIDS cPLA2 having the entirely same sequence from a sequence of Met-308 of cPLA2a of rat, a synthetic peptide having an amino acid sequence comprising 7 starting from this Met-308 (MST-TLSS—Amino Acids 1-7 of SEQ ID NO. 5) was immunized to rabbit and its serum fraction was prepared. Further, immunoglobulin (IgG) was purified from this fraction to give a final specimen. Incidentally, this stump antibody was confirmed to specifically recognize not only KIDS cPLA2 of rat but also KIDS cPLA2 of mouse.

#### Example 8

##### Analysis of Base Sequence of Intron

Analysis of intron sequences (assumed promoter region) of KIDS cPLA2 of rat and mouse was carried out by the following method.

It was investigated whether a fundamental transcription activity was present for a region ranging up to about 9 kb upstream including 5' UTR of KIDS cPLA2 of rat and mouse (a region until exon of cPLAa destroyed in knockout mouse). Firstly, there was constructed a reporter vector

where each of a sequence of about 9 kb of this region, a sequence including about 1,000 bp upstream of 5' UTR, a sequence including about 500 bp having a high homology among human being, rat and mouse and a sequence of about 700 bp including 5' UTR was integrated with the upper stream of luciferase gene. Such a reporter vector was introduced into an incubated cell strain, the supernatant liquid of the cells was prepared and its luciferase activity was measured as an index for a fundamental transcription activity. As a result, a sequence of about 700 bp including 5' UTR was found to have an especially high transcription activity.

The result is shown in FIG. 12. Further, base sequences of the animals are shown in SEQ ID NO: 12 (human being), SEQ ID NO: 13 (rat) and SEQ ID NO: 14 (mouse).

#### INDUSTRIAL APPLICABILITY

The present invention provides a novel enzyme which is presumed to be a case for cell death specific to the hippocampal dentate gyrus in stimulation by kainic acid, fit of epilepsy, etc. It is now possible to prevent the cell death by preparing an inhibitor of this enzyme.

The present invention also provides a novel enzyme having a phospholipase A2 activity and being calcium-independent.

The present invention further clarifies for the first time that, in intron, there is a function of making the initiation of transcription of RNA by an external stimulation possible. A novel function of intron in genome is elucidated and, at the same time, there is provided a new type of gene which functions as a promoter or as a regulatory gene by an external stimulation. The new type of gene functioning as a promoter or as a regulatory gene according to the present invention not only has a function as intron but also makes the expression of a stage-specific desired gene in response to an external stimulation possible and further makes the expression of the site-specific desired gene depending upon the tissue possible. Consequently, the promoter or the regulatory gene of the present invention is able to be used for the regulation of expression of gene and is applicable to transgenic animals, knockout animals, etc.

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#### SEQUENCE LISTING

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290 295 300  
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tggcatggca aaatatggta cctttatgac tcctgacttg tttggaagca aatTTTTTat 300
gggaacagtt gtaaaaaaat atgaagaaaa cccttgcat ttcttaatgg gtgtctgggg 360
cagtgccttt tctatactgt tcaacagagt tttgggagtt tctggctcac agaataaagg 420
ttctacaatg gaggaggaat tagaaaatat tacagcaaag cacattgtga gtaacgacag 480
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agagtaccaa aatgacaacc aagcaagttg ggtccatcgg atgctaattg ccttggtgag 600
tgactcagct ttattcaata cccgagaagg acgtgctggg aaggagcata acttcatggt 660
gggcttgaat ctcaacacat cgtatccact gtctcccctg agagacttca gcccccaaga 720
ttccttcgat gatgatgaac tcgacgcagc ggtagcagat ccagatgaat ttgaacgaat 780
atatgaacca ctggatgtca aaagtaaaaa gattcatggt gtagacagtg ggctcacggt 840
taacctgccg tatcccttga ttctgcgacc tcagagaggt gtggatctca tcatttcctt 900
tgacttttct gcaaggccaa gtgacaccag cctccattc aaggaacttc tgcttgacaga 960
gaagtgggct aaaatgaaca agctcccttt tccaaagatt gatccttacg tgtttgatcg 1020
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ccaaccatt atccactttg ttctggccaa catcaacttc agaaagtaca aggccccagg 1140
tgttctgagg gaaaccaaag aagagaaaga aatagctgac tttgacattt tcgatgaccc 1200
cgaatcgcca ttttcaacct tcaacttcca gtatccaaat caagcattca aaaggctaca 1260
tgatctgatg tacttcaaca cactgaacaa cattgatgtg ataaaggatg ccattggtga 1320
gagcattgaa tacagaagac agaaccctac tcgttgctct gtttcctca gtaatggtga 1380
ggcaagaaaa ttcttcaaca aggagttcct aagtaaacc acagcggagt ccatttgaat 1440
tccatgacta ctggagtca gagccacatg agagactcat cttactatgc acaagagact 1500
gactgctact cagagttgct ggggacggag gcgtgtgtta ggtgaaaatg gtgttgatta 1560
tgcaatactt ggcaacagtt tctgacagta tgaatttttt gtacataagc atagggctat 1620
atactgtatt ttaaaccatt ctcacatttt tacctgagca tttttatata tataaaaaata 1680
tcctttcctt ttataaatat ttaatagtta actcagtaaa aaaaagcttc ccattgtgtg 1740
tgaatggtat tctgaactag atttgttcat gccatgttac aa 1782

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<210> SEQ ID NO 7
<211> LENGTH: 1792
<212> TYPE: DNA
<213> ORGANISM: rat

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<400> SEQUENCE: 7

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caattgtttt agaatacaga catctatttc caggagctt tctttctggt gtctaactga 60
gaccacagat tgccagaaat aataggactt cgtttcatta taaaagaga atgagtacga 120
ccttgagtag cttgaaggaa aaggtcagcg ccgcccgggtg tcctctgcct ctcttcacct 180
gtctccatgt caaaccggac gtgtcagagc tgatgtttgc cgattgggta gaatttagtc 240
catacgaaat tggcatggca aaatatggta cctttatgac tcctgacttg tttggaagca 300
aatTTTTTat gggaacagtt gtaaaaaaat atgaagaaaa cccttgcat ttcttaatgg 360
gtgtctgggg cagtgccttt tctatactgt tcaacagagt tttgggagtt tctggctcac 420

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agaataaagg ttctacaatg gaggaggaat tagaaaatat tacagcaaag cacattgtga 480
gtaacgacag ctctgacagc gatgacgagg cccaaggacc caaaggcacc gagaatgaag 540
atgCGGaaag agagtaccaa aatgacaacc aagcaagttg ggtccatcgg atgctaattg 600
ccttggtgag tgactcagct ttattcaata cccgagaagg acgtgctggg aaggagcata 660
acttcatggt gggcttgaat ctcaacacat cgtatccact gtctcccctg agagacttca 720
gcccccaaga ttccttcgat gatgatgaac tcgacgcagc ggtagcagat ccagatgaat 780
ttgaacgaat atatgaacca ctggatgtca aaagtaaaaa gattcatggt gtagacagtg 840
ggctcacggt taacctgccg tatcccttga ttctgcgacc tcagagaggt gtggatctca 900
tcatttcctt tgacttttct gcaaggccaa gtgacaccag ccctccattc aaggaacttc 960
tgcttgacaga gaagtgggct aaaatgaaca agctcccttt tccaaagatt gatccttacg 1020
tgtttgatcg ggaaggattg aaggaatgct atgtgtttaa acctaagaat cctgatgtgg 1080
aaaaggattg cccaaccatt atccactttg ttctggccaa catcaacttc agaaagtaca 1140
aggccccagg tgttctgagg gaaaccaaag aagagaaaga aatagctgac tttgacattt 1200
tcgatgacc cgaatcgcca ttttcaacct tcaacttcca gtatccaaat caagcattca 1260
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gtaatgttga ggcaagaaaa ttcttcaaca aggagtctct aagtaaacc acagcggagt 1440
ccatttgaat tccatgacta ctggagtcca gagccacatg agagactcat cttactatgc 1500
acaagagact gactgctact cagagttgct ggggacggag gcgtgtgtta ggtgaaaatg 1560
gtgttgatta tgcaatactt ggcaacagtt tctgacagta tgaatttttt gtacataagc 1620
atagggctat atactgtatt ttaaaccatt ctcacatttt tacctgagca tttttatata 1680
tataaaaata tcctttcctt ttataaatat ttaatagtta actcagtaaa aaaaagcttc 1740
ccattgtgtg tgaatgttat tctgaactag atttgttcat gccatgttac aa 1792

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<210> SEQ ID NO 8
<211> LENGTH: 441
<212> TYPE: PRT
<213> ORGANISM: mouse

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<400> SEQUENCE: 8

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Met Ser Met Thr Leu Ser Ser Leu Lys Glu Lys Val Asn Ala Ala Arg
1           5           10           15
Cys Pro Leu Pro Leu Phe Thr Cys Leu His Val Lys Pro Asp Val Ser
          20           25           30
Glu Leu Met Phe Ala Asp Trp Val Glu Phe Ser Pro Tyr Glu Ile Gly
          35           40           45
Met Ala Lys Tyr Gly Thr Phe Met Ala Pro Asp Leu Phe Gly Ser Lys
          50           55           60
Phe Phe Met Gly Thr Val Val Lys Lys Tyr Glu Glu Asn Pro Leu His
65           70           75           80
Phe Leu Met Gly Val Trp Gly Ser Ala Phe Ser Ile Leu Phe Asn Arg
          85           90           95
Val Leu Gly Val Ser Gly Ser Gln Asn Lys Gly Ser Thr Met Glu Glu
          100          105          110
Glu Leu Glu Asn Ile Thr Ala Lys His Ile Val Ser Asn Asp Ser Ser
          115          120          125
Asp Ser Asp Asp Glu Ala Gln Gly Pro Lys Gly Thr Glu Asn Glu Glu

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130	135	140																		
Ala	Glu	Lys	Glu	Tyr	Gln	Ser	Asp	Asn	Gln	Ala	Ser	Trp	Val	His	Arg					
145					150				155						160					
Met	Leu	Met	Ala	Leu	Val	Ser	Asp	Ser	Ala	Leu	Phe	Asn	Thr	Arg	Glu					
				165					170					175						
Gly	Arg	Ala	Gly	Lys	Val	His	Asn	Phe	Met	Leu	Gly	Leu	Asn	Leu	Asn					
			180					185					190							
Thr	Ser	Tyr	Pro	Leu	Ser	Pro	Leu	Arg	Asp	Phe	Ser	Ser	Gln	Asp	Ser					
		195					200					205								
Phe	Asp	Asp	Glu	Leu	Asp	Ala	Ala	Val	Ala	Asp	Pro	Asp	Glu	Phe	Glu					
	210					215					220									
Arg	Ile	Tyr	Glu	Pro	Leu	Asp	Val	Lys	Ser	Lys	Lys	Ile	His	Val	Val					
225					230					235					240					
Asp	Ser	Gly	Leu	Thr	Phe	Asn	Leu	Pro	Tyr	Pro	Leu	Ile	Leu	Arg	Pro					
				245					250					255						
Gln	Arg	Gly	Val	Asp	Leu	Ile	Ile	Ser	Phe	Asp	Phe	Ser	Ala	Arg	Pro					
			260					265					270							
Ser	Asp	Thr	Ser	Pro	Pro	Phe	Lys	Glu	Leu	Leu	Leu	Ala	Glu	Lys	Trp					
		275					280					285								
Ala	Lys	Met	Asn	Lys	Leu	Pro	Phe	Pro	Lys	Ile	Asp	Pro	Tyr	Val	Phe					
	290					295					300									
Asp	Arg	Glu	Gly	Leu	Lys	Glu	Cys	Tyr	Val	Phe	Lys	Pro	Lys	Asn	Pro					
305					310					315					320					
Asp	Val	Glu	Lys	Asp	Cys	Pro	Thr	Ile	Ile	His	Phe	Val	Leu	Ala	Asn					
				325					330					335						
Ile	Asn	Phe	Arg	Lys	Tyr	Lys	Ala	Pro	Gly	Val	Leu	Arg	Glu	Thr	Lys					
			340					345					350							
Glu	Glu	Lys	Glu	Ile	Ala	Asp	Phe	Asp	Ile	Phe	Asp	Asp	Pro	Glu	Ser					
		355					360					365								
Pro	Phe	Ser	Thr	Phe	Asn	Phe	Gln	Tyr	Pro	Asn	Gln	Ala	Phe	Lys	Arg					
	370					375					380									
Leu	His	Asp	Leu	Met	Tyr	Phe	Asn	Thr	Leu	Asn	Asn	Ile	Asp	Val	Ile					
385					390					395					400					
Lys	Asp	Ala	Ile	Val	Glu	Ser	Ile	Glu	Tyr	Arg	Arg	Gln	Asn	Pro	Ser					
				405					410					415						
Arg	Cys	Ser	Val	Ser	Leu	Ser	Asn	Val	Glu	Ala	Arg	Lys	Phe	Phe	Asn					
			420					425					430							
Lys	Glu	Phe	Leu	Ser	Lys	Pro	Thr	Val												
	435						440													

<210> SEQ ID NO 9  
 <211> LENGTH: 1861  
 <212> TYPE: DNA  
 <213> ORGANISM: mouse

<400> SEQUENCE: 9

gaaaatgatt tgcttagata tgttatcttg aataactttt atctgtgcc catgcctatg 60  
 tatttaaagc catctcttct tttcttatgt ttgtggacag aggatgagca tgaccctgag 120  
 tagtttgaag gaaaaggcca atgccgcccc gtgtcctttg cctctcttca cgtgtctcca 180  
 cgtaaacct gatgtgtcag agctgatgtt tgccgattgg gtggaattta gtccatatga 240  
 gattggcatg gcaaaatatg gtacctttat ggctcctgac ctatttgaa gcaagttttt 300  
 tatgggaaca gttgtaaaaa aatatgaaga aaacccttg catttcttga tgggtgtctg 360



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gggcagtgcc ttttctatac tgttcaacag agttttggga gtttctggct cacagaataa 420
aggctctaca atggaagagg aattagaaaa tattacagca aagcacatcg tgagtaatga 480
cagctccgac agtgatgatg aggctcaagg acccaaaggc accgagaatg aagaagctga 540
aaaagagtac caaagcgaca accaagcaag ttgggtccat cggatgctaa tggccttggt 600
gagcgactcg gctttattca atacccgaga aggacgtgcc ggaaaggtgc ataacttcat 660
gctgggcttg aatctcaaca catcatatcc actgtctccc ctgagagact tcagctctca 720
ggattccttc gatgacgagc tcgacgcagc ggtagcagat ccagatgaat ttgaacgaat 780
atatgaacca ctggatgtca aaagtaagaa gattcatgtg gtagatagtg ggctcacatt 840
taacctgcca tatcccttga ttcttcgacc tcagagaggt gtggatctta tcatctcctt 900
tgacttttct gcaaggccga gtgacaccag tcccccttcc aaggaacttc tgcttgacaga 960
gaagtgggcy aaaatgaaca agcttccctt tccaaagatc gatccttatg tgtttgatcg 1020
ggaaggatta aaggaatgct atgtttttaa acctaagaat cctgatgtgg agaaggattg 1080
cccaaccatt atccactttg ttctggccaa catcaacttc agaaagtaca aggccccagg 1140
tgttctaagg gaaaccaaag aagagaaaga aattgctgac tttgacattt ttgatgaccc 1200
cgaatcgcca ttttcaacct tcaactttca gtatcccaat caagcattca aaaggcttca 1260
cgatttgatg tacttcaaca cactgaacaa cattgatgtg ataaaggatg ccattggtga 1320
gagcattgaa tacagaagac agaaccatc tcgttgctct gtttccctca gtaatgttga 1380
agcaagaaaa ttcttcaata aggagtttct aagtaaacc actgtgtaat ttctgtgctg 1440
ggatgatcaa gccatttgaa ttccatgaca atttgagttc agaagacatt agaggtcac 1500
ttactatgca gaagagactg gctgctactc aaagttgtgg agatttagcc atgtgttagg 1560
tgaaaatgat gttgattatg taatacttag caacagtttc tgacagtatg aattttttga 1620
cattagcata gagctatata ctgtatttta aacattcctc acatttttta cctgtacttt 1680
ttatataaat atgacatgtc ttttcttttg aaaatattta atagtttaac tcagtaaagg 1740
agacttccca ttgtgtgtga atgttattct gaactagatt tgttcatgcc atgttacaac 1800
actattttta tttaaatggt tatatttaca catacgaaat aaatactttg ctgtacaaat 1860
t 1861

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<210> SEQ ID NO 10
<211> LENGTH: 1860
<212> TYPE: DNA
<213> ORGANISM: mouse

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<400> SEQUENCE: 10

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agttgtttta aaatacacac atctttttcc ctggaacttt atttctgttg tctacttgag 60
accacagatt tccaggaata ataggacttc atttcattaa ggatgagcat gaccctgagt 120
agtttgaagg aaaagggtcaa tgccgcccgg tgcctttgac ctctcttcac gtgtctccac 180
gtcaaacctg atgtgtcaga gctgatgttt gccgattggg tggaaattag tccatagag 240
attggcatgg caaaatatgg tacctttatg gctcctgacc tatttggaag caagtttttt 300
atgggaacag ttgtaaaaaa atatgaagaa aacccttgc atttcttgat ggggtgtctgg 360
ggcagtgcct tttctatact gttcaacaga gttttgggag tttctggctc acagaataaa 420
ggctctacaa tggaagagga attagaaaat attacagcaa agcacatcg gagtaatgac 480
agctccgaca gtgatgatga ggctcaagga cccaaaggca ccgagaatga aagaagctgaa 540
aaagagtacc aaagcgacaa ccaagcaagt tgggtccatc ggatgctaat ggccttggtg 600

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agcgactcgg ctttattcaa tacccgagaa ggacgtgccg gaaaggtgca taacttcacg 660
ctgggcttga atctcaacac atcatatcca ctgtctcccc tgagagactt cagctctcag 720
gattccttcg atgacgagct cgacgcagcg gtagcagatc cagatgaatt tgaacgaata 780
tatgaaccac tggatgtcaa aagtaagaag attcatgtgg tagatagtgg gctcacattt 840
aacctgccat atcccttgat tcttcgacct cagagagggtg tggatcctat catctccttt 900
gacttttctg caaggccgag tgacaccagt cccctttca aggaacttct gcttcgagag 960
aagtgggcca aatgaacaa gcttcccttt ccaaagatcg atccttatgt gtttgatcgg 1020
gaaggattaa aggaatgcta tgtttttaa cctaagaatc ctgatgtgga gaaggattgc 1080
ccaaccatta tccactttgt tctggccaac atcaacttca gaaagtacaa ggccccaggt 1140
gttctaaggg aaaccaaaga agagaaagaa attgctgact ttgacatttt tgatgacccc 1200
gaatcgccat tttcaacctt caactttcag tatcccaatc aagcattcaa aaggcttcac 1260
gatttgatgt acttcaacac actgaacaac attgatgtga taaaggatgc cattggtgag 1320
agcattgaat acagaagaca gaaccatct cgttgctctg tttccctcag taatggtgaa 1380
gcaagaaaat tcttcaataa ggagtttcta agtaaacca ctgtgtaatt tctgtgctgg 1440
gatgatcaag ccatttgaat tccatgacaa tttgagttca gaagacatta gaggtcatct 1500
tactatgcag aagagactgg ctgctactca aagtgtgga gatttagcca tgtgttaggt 1560
gaaaatgatg ttgattatgt aatacttagc aacagtttct gacagtatga attttttgac 1620
attagcatag agctatatac tgtattttaa acattcctca cattttttac ctgtactttt 1680
tatataaata tgacatgtct tttcttttga aaatatttaa tagtttaact cagtaaagga 1740
gacttcccat tgtgtgtgaa tgttattctg aactagattt gttcatgcca tgttacaaca 1800
ctatttttat ttaaagtgtt atatttacac atacgaaata aatactttgc tgtacaaatt 1860

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<210> SEQ ID NO 11
<211> LENGTH: 1966
<212> TYPE: DNA
<213> ORGANISM: mouse

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<400> SEQUENCE: 11

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agttgtttta aaatacacac atctttttcc ctggaacttt atttctgttg tctacttgag 60
accacagatt tccaggaata ataggacttc atttcattat aaaatgaaaa tgatttgctt 120
agatatgtta ttttgaataa cttttatctg tgccccatgc ctatgtattt aaagccatct 180
cttcttttct tatgtttgtg gacagaggat gagcatgacc ctgagtagtt tgaaggaaaa 240
ggccaatgcc gcccggtgtc ctttgccctct cttcaagtgt ctccacgtca aacctgatgt 300
gtcagagctg atgtttgccg attgggtgga atttagtcca tatgagattg gcatggcaaa 360
atatggtacc tttatggctc ctgacctatt tggaagcaag ttttttatgg gaacagttgt 420
aaaaaatat gaagaaacc ccttgcattt cttgatgggt gtctggggca gtgccttttc 480
tatactgttc aacagagttt tgggagtttc tggtcacag aataaaggct ctacaatgga 540
agaggaatta gaaaatatta cagcaaagca catcgtgagt aatgacagct ccgacagtga 600
tgatgaggct caaggacca aaggcacca gaatgaagaa gctgaaaaag agtaccacaa 660
cgacaacca gcaagtggg tccatcggat gctaattggc ttgggtgagc actcggcttt 720
attcaatacc cgagaaggac gtgccgaaa ggtgcataac ttcagctgg gcttgaatct 780
caacacatca tatccactgt ctcccctgag agacttcagc tctcaggatt ccttcgatga 840

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cgagctcgac gcagcggtag cagatccaga tgaatttgaa cgaatatatg aaccactgga 900
tgtcaaaagt aagaagattc atgtggtaga tagtgggctc acatttaacc tgccatatcc 960
cttgattctt cgacctcaga gaggtgtgga tcttatcatc tcctttgact tttctgcaag 1020
gccgagtgc accagtcccc ctttcaagga acttctgctt gcagagaagt gggcgaaaat 1080
gaacaagctt ccctttccaa agatcgatcc ttatgtgttt gatcgggaag gattaaagga 1140
atgctatggt tttaaacctc agaatcctga tgtggagaag gattgcccac ccattatcca 1200
ctttgttctg gccaacatca acttcagaaa gtacaaggcc ccagggtgtc taagggaaac 1260
caaagaagag aaagaaattg ctgactttga ctttttgat gaccccgat cgccattttc 1320
aaccttcaac tttcagtatc ccaatcaagc attcaaaagg cttcacgatt tgatgtactt 1380
caacacactg aacaacattg atgtgataaa ggatgccatt gttgagagca ttgaatacag 1440
aagacagaac ccactctggt gctctgtttc cctcagtaat gttgaagcaa gaaaattctt 1500
caataaggag tttctaagta aaccactgt gtaatttctg tgctgggatg atcaagccat 1560
ttgaattcca tgacaatttg agttcagaag acattagagg tcactttact atgcagaaga 1620
gactggctgc tactcaaagt tgtggagatt tagccatgtg ttaggtgaaa atgatgttga 1680
ttatgtaata cttagcaaca gtttctgaca gtatgaattt tttgacatta gcataagact 1740
atatactgta ttttaaacat tcctcacatt tttacctgt actttttata taaatagac 1800
atgtcttttc ttttgaaaat atttaatagt ttaactcagt aaaggagact tcccattgtg 1860
tgtgaatggt attctgaact agatttgctc atgccatggt acaacactat ttttatttaa 1920
atgtttatat ttacacatac gaaataaata ctttctgta caaatt 1966

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<210> SEQ ID NO 12
<211> LENGTH: 560
<212> TYPE: DNA
<213> ORGANISM: Human

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<400> SEQUENCE: 12

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taattcattt caatgatgta aagattttga atgtgtgagg aagtgtttt gtattccttt 60
tctctggaaa aaaaaaaaaa aaaaaaatt cacattttta cccttaactg cccattccct 120
ccaagaatgg taacattttt agatgaggaa gaatgaagtt tgccatgaata gagtcaagaa 180
aggaagggga tcgcatagaa cagactcgct tgatgcatga ttgcattgat gtttcggtga 240
agataaagca gaggagcgcc tgtgacaggg agtccagggg ctaagtttct tccaggctcc 300
acagttgcta attcattctc cagttcagat gtagacatat aatctagagt tatgattatt 360
ttttaaataga agatagttac ttccatagag cttatttttt gttgttcatt caggacctag 420
taatttctag aagtaataag acttattttt attataaagt tataagattt tgattggaag 480
tactattttg aatagcattc tttctgtgct tgtttataaa tttaaagtca tctttttctt 540
tcttctgtgg acagagaatg 560

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<210> SEQ ID NO 13
<211> LENGTH: 667
<212> TYPE: DNA
<213> ORGANISM: rat

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<400> SEQUENCE: 13

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```

taaaaaagt aatatttagg tgagatggta agactatgca ttgcttttga ggggatgtg 60
agttcagtag tcagcaccta catctggcag cgcacaactg cctgtaactc cagctttagg 120
aggagccgat acctctggcc tttatgaaca cctacactca catgcatata tccacacaca 180

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gataatatac atatgcatat tttacttttt atattcatat tttaaaataa tagaagtggg 240
agaaaaata ctctttgcct agtaagagtc aaataaggaa atggatcata ggaaacaaat 300
gtacttgatg tgtcaccaga ggggtgacatt tcatctgaag ataaagcagg agagacggga 360
caacctgtgc caggacacc agctgagaga attagttccc agaactatag ctgccaaatc 420
ttccccact tcaaagtgtg acacagtccc cagagattca attgttttag aatacagaca 480
tctatttcca gggagctttc tttctgttgt ctaatcgaga ccacagattg ccagaaataa 540
taggacttcg tttcattata aaaaggcaag cgatttggtt agacatatta tttcaaatag 600
cttttatctg tgtccatgtc tatgtattta aagccacctt attctttttg tgtgtgtgtg 660
tgaaaag 667

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<210> SEQ ID NO 14
<211> LENGTH: 739
<212> TYPE: DNA
<213> ORGANISM: mouse
<220> FEATURE:
<221> NAME/KEY: base
<222> LOCATION: 496
<223> OTHER INFORMATION: n = a, g, c, t, unknown, or other
<220> FEATURE:
<221> NAME/KEY: base
<222> LOCATION: 497
<223> OTHER INFORMATION: n = a, g, c, t, unknown, or other

<400> SEQUENCE: 14

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```

tggtaatggt gtgaatagaa gtgctattta ggtgagatcg tgaatacaag tgatattaaa 60
agcaggaagg aggaggtttt cgctcctcag cagataagcc catgcactgc tcttgtgggg 120
acatgagttc aacaaccagc acctatgtct gacagctcat aactacctgt aattccagat 180
tcaggaggtg ccaatacctc tggcctctgt gaatatctgc actcacaatc atatatccac 240
acacagatac atattcatat acatatttta ttttttatat tcacatttta aaataataaa 300
ttgaaatggt agaagaacac tctttgcctc ataagagaca aataaggaaa tggacatgg 360
gaaacaaatg gacttgatgt gtcaccagag ggtgacattt catctgaaga taaagcaggg 420
gagaaaggac agctgtgcca gggaacgcca gctgagagaa ctagttctca aactctagt 480
tgccaaatct tctcnnctt caaatgttga cacagtcttc agagattcag ttgttttaaa 540
atacacacat cttttccctt ggaactttat ttctgttgtc tacttgagac cacagatttc 600
caggaataat aggacttcat ttcattataa aatgaaaatg atttgcttag atatgttatt 660
ttgaataact tttatctgtg ccccatgcct atgtatttaa agccatctct tcttttctta 720
tgtttggga cagaggatg 739

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The invention claimed is:

1. An isolated calcium-independent phospholipase A2<sup>55</sup> consisting of the amino acid sequence as set forth in SEQ ID NO: 1, NO: 5 or NO: 8 of the Sequence Listing.

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